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December 1998

Characteristics and Causes of Texas Marine Strandings

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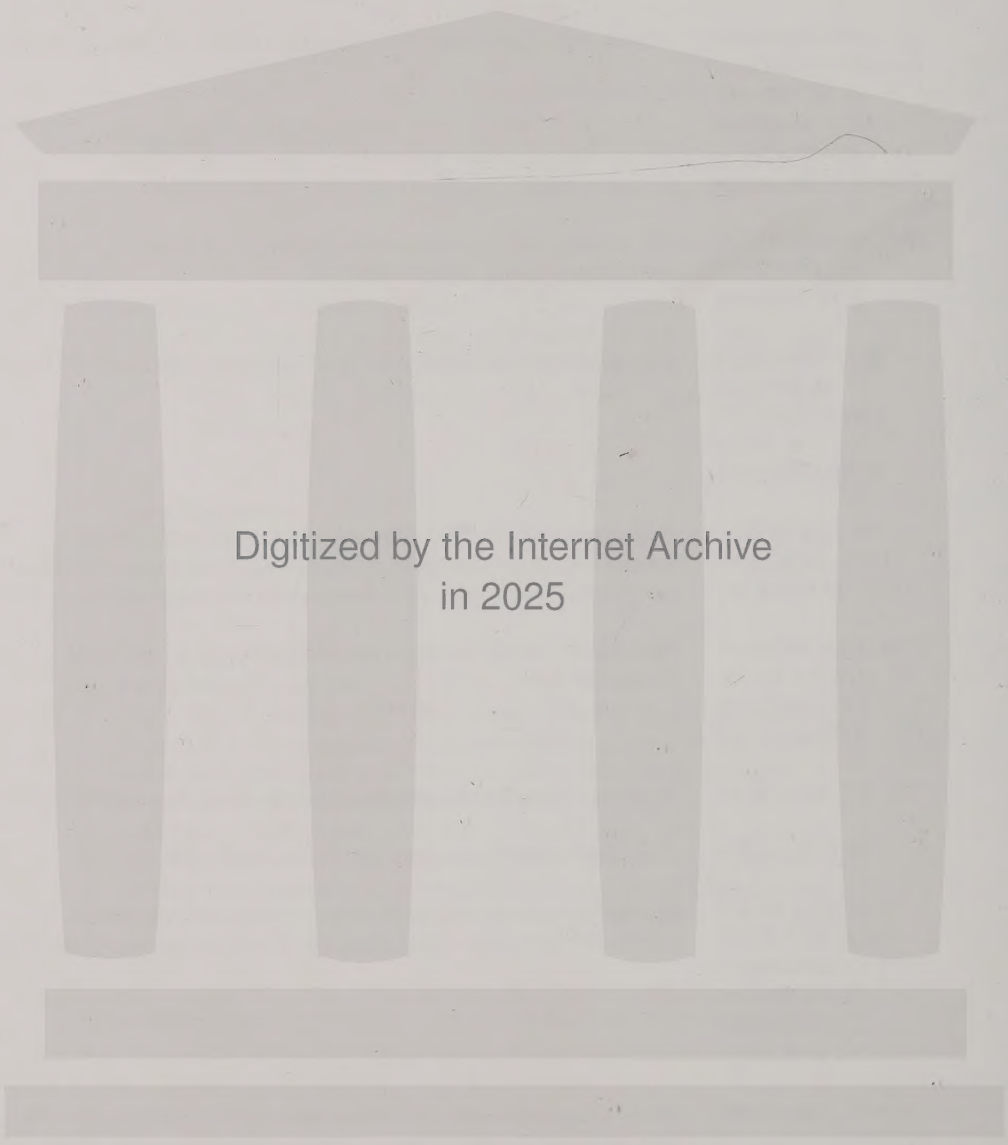
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INTRODUCTION

Three major mass mortality events occurred on the upper Texas coast during 1994, from January through the second week of May. These events were distinguished by unusually large numbers of dead dolphins, sea turtles, and fishes washing ashore on Texas beaches. The beach stranding of dead animals began in January with bottlenose dolphins. By the end of March, 142 dolphins had washed ashore as compared to about 40 expected. By the latter part of April, dolphin mortalities declined but stranding of dead and comatose sea turtles increased. By the end of April, at least 127 sea turtles had stranded on the Texas coast since the beginning of the year, about double the expected number. Then, during May and June, a third mortality event began with a massive fish kill and more turtle deaths. By the middle of May, mortalities of all species as indicated by beach strandings returned to within expected levels. Nevertheless, 1994 stood out as a record year of marine mass mortalities in the northwestern Gulf of Mexico.

Due to the magnitude and across-class nature of the mass mortalities, and concurrent with living marine resource responsibilities, the National Oceanographic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS) and the State of Texas Parks and Wildlife Department convened a post-event conference of experts in Galveston, Texas. The purpose of the conference was to characterize the events and discuss probable causes of the deaths of dolphins, sea turtles, and fishes and their association with coincident harmful algal blooms. This NOAA Technical Report details much of the information gathered by the conference experts, based upon what was known at that time.

From the 1994 conference, Paula Bontempi and Carrie Lyons report on the offshore hydrographic and phytoplankton transects across the Texas-Louisiana shelf provided by the LATEX Data Office at Texas A&M University (p. 1–12). The physical conditions leading to the 1994 events appeared to be similar to conditions of a June 1984 harmful algal bloom which was linked to extensive kills of two demersal fish species, threadfin and croaker (Harper and Guillen, 1989). Similarities between the incidents included a high volume of freshwater runoff, strong prevailing onshore winds, stratification of surface and bottom waters, and hypoxia. William Wardle, Winston Denton, and Donald Harper compare physical and biological conditions between the 1984 and 1994 events (p. 33–39).

Phytoplankton blooms, low oxygen levels in bottom waters, and fish kills were observed to coincide during 1994. Karen Steidinger, Dean Stockwell, Earnest Truby, William Wardle, Quay Dortch, and Frances Van Dolah describe sampled associations of dinoflagellate blooms with mortality of demersal fishes (p. 13–17). Occurrence of the numerically-dominant toxic dinoflagellate identified as *G. sanguineum* (=*G. splendens*) is elaborated by Randy Robichaux, Quay Dortch, and John Wrenn (p. 19–25). The extent of the May and June 1994 fish kills are described by Winston Denton, Dave Buzan, Jerry Mambretti, Ken Rice, and Karen Quiñonez (p. 27–31). Frances Van Dolah, Gregory Doucette, Tod Leighfield, and Karen Steidinger report on toxicity assays of natural water samples with *G. sanguineum* present (p. 41–45).

The deaths of dolphins and sea turtles began before mortality of fishes. Graham Worthy describes the mortality event involving the bottlenose dolphin, *Tursiops truncatus,* and its association with the disease morbillivirus (p. 47–55). He emphasizes the difficulty in finding a definitive cause due to the advanced state of decomposition of the stranded carcasses (condition code 4). Morbillivirus has been associated with mass deaths of dolphins before, including the deaths of 10,000+ dolphins in the Mediterranean Sea in 1990-91. However, the offshore area in which most of the 1994 dolphin mortalities occurred also coincided with the area in which toxic dinoflagellates were found.

Donna Shaver describes record setting sea turtle strandings and association with the shrimp fishery (p. 57–72). A significant relationship between sea turtle strandings and nearshore shrimping effort has continued even after the implementation of Turtle Excluder Devices (TED's) in shrimp trawls (Caillouet et al., 1996). The implication is that TED's are not infallible and that technical problems may occur even when TED's are installed in commercial shrimp nets. During the summer of 1994, technical problems in TED's were observed and corrected thereafter by NMFS. Also, TED's may be illegally disabled by a shrimp vessel operator and only enhanced enforcement seems to lessen this problem.

During April and May of 1994, six comatose sea turtles were found stranded and all had reduced heart rates and exhibited poor muscular control. Five of the six turtles could not breathe on their own, so their lungs were mechanically ventilated. Resuscitation attempts failed and all of these turtles died within 1 to 4 days. The possibility remains that exposure to waters with blooms of toxic dinoflagellates may have affected these and other sea turtles and increased mortality due to capture in shrimp trawls, even when operational TED's

were present. Ann Colbert, M. Fulton, J. Landsberg, J. Newton, J. Cullen, and G. Scott report on analyses of water and animal tissue samples and essentially eliminate as a causative factor man-made toxins such as pesticides (p. 73–79). Andrea Cannon confirms that the primary cause of sea turtle mortality was not evident by gross necropsy (p. 81–85).

Harmful algae, low oxygen, and shrimp trawling are implicated as factors, acting together or singly, that led to the mass mortality of marine animals in waters off the upper Texas coast and western Louisiana during 1994. The synergism among these factors and other causes could have accounted for the higher than normal mortality rates encountered. It is also likely that exposure to algal biotoxins and unfavorable environmental conditions made these marine animals more susceptible to succumbing to disease and other stresses.

Five years after the record setting mass mortality events of 1994, the record stills stands. Other mortality events in the Gulf of Mexico and investigations since 1994 yield some insights. In separate events during 1996, manatee deaths in Florida and dolphin deaths in Mississippi were associated with red tide toxic algal blooms. Also, it is now known that after the 1993 Mississippi River flood, the summer hypoxic zone on the Louisiana shelf, sometimes called the "Dead Zone," doubled in area up to 7,000 square miles (Rabalais and Turner, in press).

Nonetheless, the evidence for cause-and-effect implicating harmful algal blooms and hypoxia in the 1994 mass mortality cases remains circumstantial. Yet, the perturbations of Mississippi River discharge, such as over-enrichment of nitrates, presence of toxic dinoflagellates, and bottom-water hypoxia (Rabalais et. al., 1996), continue in Louisiana and Texas shelf waters and the risk is high that mass mortality events among the same species will return. A follow-up conference to evaluate the state of knowledge of mass mortality events among marine animals in the Gulf of Mexico would be of benefit to science and management. Better yet would be a dedicated NOAA-funded program to develop credible information on cause-and-effect relationships leading to mass mortalities of northwestern Gulf shelf species.

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An Assessment of Oxygen, Salinity, and Phytoplankton Distributions Near an Area off Sabine Pass, Texas, Characterized by Demersal Fish and Marine Mammal Mortalities

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ABSTRACT

Three years of springtime hydrographic and phytoplankton data were collected on transects across the Texas-Louisiana continental shelf from 1992–94 as part of LATEX (Louisiana-Texas Shelf Physical Oceanography Program). Waters located offshore of the LATEX study area, about 3 km from the Sabine River mouth, were marked by a mortality incident involving demersal fishes, dolphins, and sea turtles in April 1994. The LATEX transect near the affected area originates slightly southwest (16 km) from the Sabine River mouth and follows along 94°W longitude. Possible causes of the 1994 fish kill were compared with proposed causes of a fish kill that occurred in a similar area on the Texas-Louisiana shelf in 1984. Harper and Guillen (1989) speculated that the cause of the June 1984 fish kill was linked to hydrogen sulfide production stemming from anoxic conditions. Anoxic conditions were said to be caused by the influx of fresh Mississippi River water and resultant water column stability, in conjunction with the spring phytoplankton bloom and aerobic decomposition of organic matter produced in the bloom. Dissolved oxygen and salinity data from the first week of May 1994 did not indicate hypoxia or hydrogen sulfide production had taken place. No unusual trends were found in hydrographic and phytoplankton data from 1994 when compared with May 1992 and May 1993 data. The presence of toxic algal species was not detected, nor was a phytoplankton bloom identified in 1994. During the spring of 1992, 1993, and 1994, dominant phytoplankton on the inner shelf along 94°W longitude included chain-forming diatoms, dinoflagellates of the family Gymnodiniaceae, and several cryptomonad species. It is unlikely that phytoplankton were the cause of the 1994 fish kill.

Introduction

Mortality events involving several species of sea turtles, dolphins, and demersal fishes occurred in the northwest Gulf of Mexico in late April 1994. A second mortality incident occurred during June 1994. Both events first appeared near Sabine Pass at the border of Texas and Louisiana and extended west to Freeport, Texas (Fig. 1). The first event was marked by brown-colored patches of water 2–3 km offshore of Sabine Pass near Cameron, Louisiana. As increasing numbers of dead hardhead and gafftopsail catfish (*Arius felis* and *Bagre marinus*, respectively) washed up on the beaches, and sea turtle and dolphin strandings increased in these areas, state and federal agencies were called in to investigate.

Initial theories formulated by the National Marine Fisheries Service and Texas Parks and Wildlife Department were similar to those stated in a study done by Harper and Guillen (1989) regarding a fish kill that occurred off the Texas-Louisiana coast in June 1984. The 1984 fish kill not only occupied the same area, but targeted benthic and demersal fishes and invertebrates as during April 1994. The most supported cause of the 1984 fish kill was a bloom of the dinoflagellate *Gymnodinium splendens*, which was related to increased springtime Mississippi-Atchafalaya river runoff (Harper

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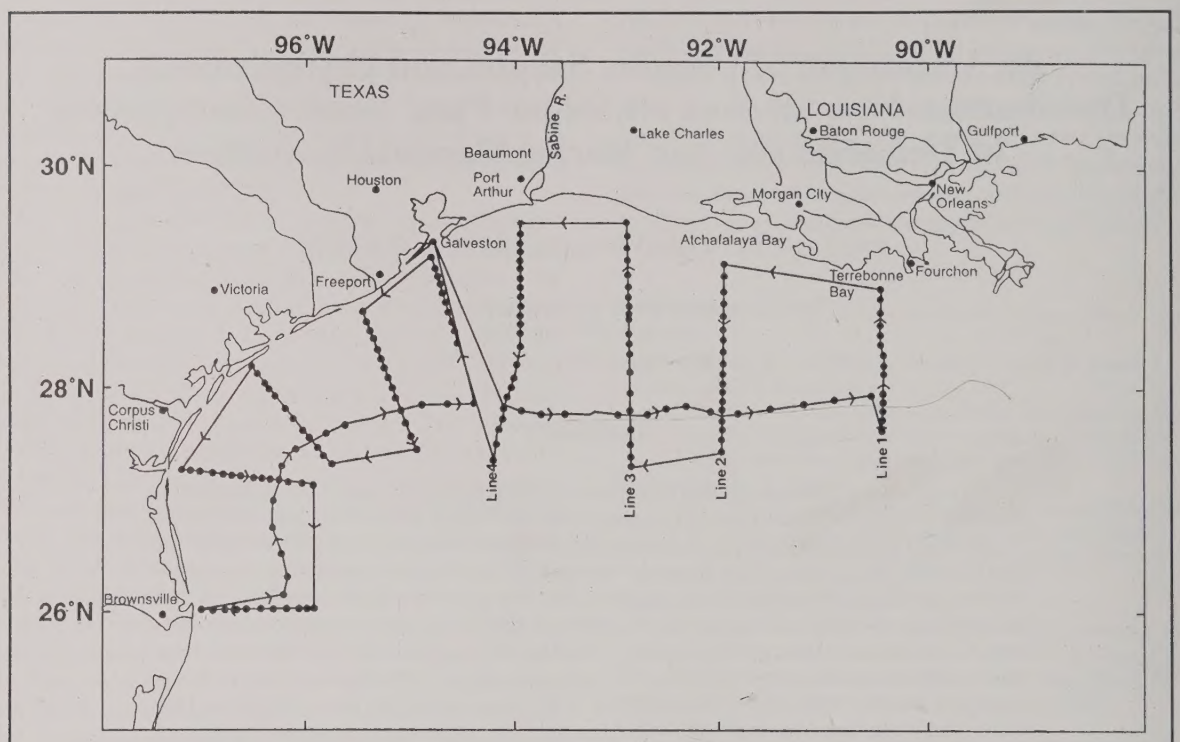


Figure 1

Map of the Texas-Louisiana continental shelf with LATEX A cruise track and station locations. Station 071 location is indicated by a star on Line 4. The May 1992 cruise included only the first four easternmost transects.

and Guillen, 1989). The 1984 bloom and low-salinity water mass were carried to the area off Sabine Pass by downcoast alongshore currents which prevail near shore during the spring (Cochrane and Kelly, 1986). It was speculated that Mississippi-Atchafalaya river runoff lowered salinities and delivered nutrients onto the shelf, supporting the phytoplankton bloom. As phytoplankton die and settle to the bottom, decay processes occur as bacterial respiration takes place (Harper and Guillen, 1989), decreasing oxygen levels. Harper and Guillen (1989) stated that oxygen depletion and stratification resulting from freshwater flow may have facilitated the 1984 fish kill, and water column stratification was intensified due to calm weather. The presence of hydrogen sulfide associated with anaerobic silt in the area of the fish kill off Galveston, Texas, may have affected demersal fish and benthic invertebrates and caused the fish kill (Harper and Guillen, 1989).

In 1994, several possible causes for the observed fish kill and turtle and dolphin strandings were explored, including the 1984 hypotheses. Other potential causes included the presence of a toxic dinoflagellate bloom, effects of shrimp trawling, toxic wastes, pesticides associated with agricultural and river runoff, menhaden purse seining, and the production of hydrogen sulfide stemming from anoxic conditions (brought on by freshwater runoff, resulting water column stability, and decomposition of fluxed organic materials). While initial sample collection and tests were being run on fish, turtles, dolphins, and phytoplankton in the affected area offshore from Sabine Pass, scientists working on the LATEX program were contacted for dissolved oxygen, salinity, and phytoplankton data near the initial mortality incident area. The purpose of this paper is to examine theories of previous mortality events while establishing oceanographic conditions within the vicinity of the fish kill area.

Materials and Methods

The Texas-Louisiana shelf was surveyed in late April/early May 1994 during a spring LATEX A (Texas-Louisiana Shelf Circulation and Transport Processes Study) hydrographic cruise at the time of the initial mortality incident off of Sabine Pass. The spring 1994 LATEX A cruise was the third spring cruise in a series of seasonal hydrographic surveys covering the shelf since 1992.

The LATEX A database included three consecutive years of hydrographic information from 1992 through 1994. The spring 1992 and 1994 hydrographic cruises took place aboard the R/V *Gyre*, and the spring 1993 cruise was aboard the R/V *J.W. Powell*.

The sampling grid was arranged in several cross-shelf transects covering the area from Terrebonne Bay, Louisiana, to Brownsville, Texas (Fig. 1). The transect near the fish kill area, Line 4, began at the 10 m isobath along 94°W longitude, approximately 12 km seaward of the fish kill. Discolored water associated with the area of the mortality incident was found about 2–3 km offshore of Sabine Pass. The LATEX A data is from an area near, but not within, the mortality incident area.

Twenty-eight stations were occupied on the transect line along 94°W during each cruise (Fig. 1). At each station, a Sea-Bird Electronics-911*plus*[1] CTD was used to collect continuous profiles of temperature, conductivity, dissolved oxygen, transmissometry (SeaTech 2000 m), fluorescence (SeaTech 3000 m), optical backscatterance (D&A Instruments OBS-3), and downwelling irradiance (Biospherical Instruments QSP-200L) with depth. An altimeter (Datasonics PSA-900) was attached to the CTD-Rosette (General Oceanics 12 place) frame. Coupled with the CTD package were twelve 10-L General Oceanics Lever Action Niskin Bottles on the Rosette. An Acoustic Doppler Current Profiler (ADCP) recorded data continuously as well. At predetermined stations, continuous data profiles were collected by the CTD on the downcast, and Niskin bottles were tripped on the upcast at various depths. Discrete water samples were drawn from the Niskin bottles to measure a host of hydrographic parameters, including dissolved oxygen and salinity. Levels of dissolved oxygen were determined by micro-Winkler titration (Carpenter, 1965) and salinity determined by a Guildline Autosal onboard the ship.

Aside from the host of hydrographic data collected during LATEX A surveys, preserved phytoplankton samples and net tows were collected at the same locations as hydrographic data (near the area of the initial mortality incident). Phytoplankton data were for a complementary program supported by the Office of Naval Research (ONR).

During the May 1992 (92A) and May 1993 (93E) cruises, five stations along Line 4 were chosen for microscopic enumeration and taxonomic identification of phytoplankton. A 250 ml water sample was collected from the surface and chlorophyll maximum at each station. Samples were preserved in a 1% glutaraldehyde solution and stored at 5°C until enumerated according to the Utermöhl technique (Utermöhl, 1958). Glutaraldehyde was chosen as a preservative for the phytoplankton after evaluation of the ONR study objectives and consultation with Greta A. Fryxell at Texas A&M University. For the spring 1994 cruise, station 071 on Line 4 was examined (Fig. 1). This station is located about 12 km seaward from the mouth of the Sabine River. Surface and chlorophyll maximum phytoplankton samples were enumerated, and a vertical net tow sample (27μm mesh) was examined for phytoplankton species composition. Phytoplankton were identified to species level wherever possible, and raw count data was converted to abundance numbers (cells·L^{-1}). Springtime dissolved oxygen, salinity, current flows, and surface phytoplankton distributions were reviewed for anomalous events during the April/May 1994 cruise period on Line 4 (30 April–1 May), after being compared with April/May 1992 (1–3 May) and 1993 (2–3 May) Line 4 cruise data.

Results and Discussion

Circulation

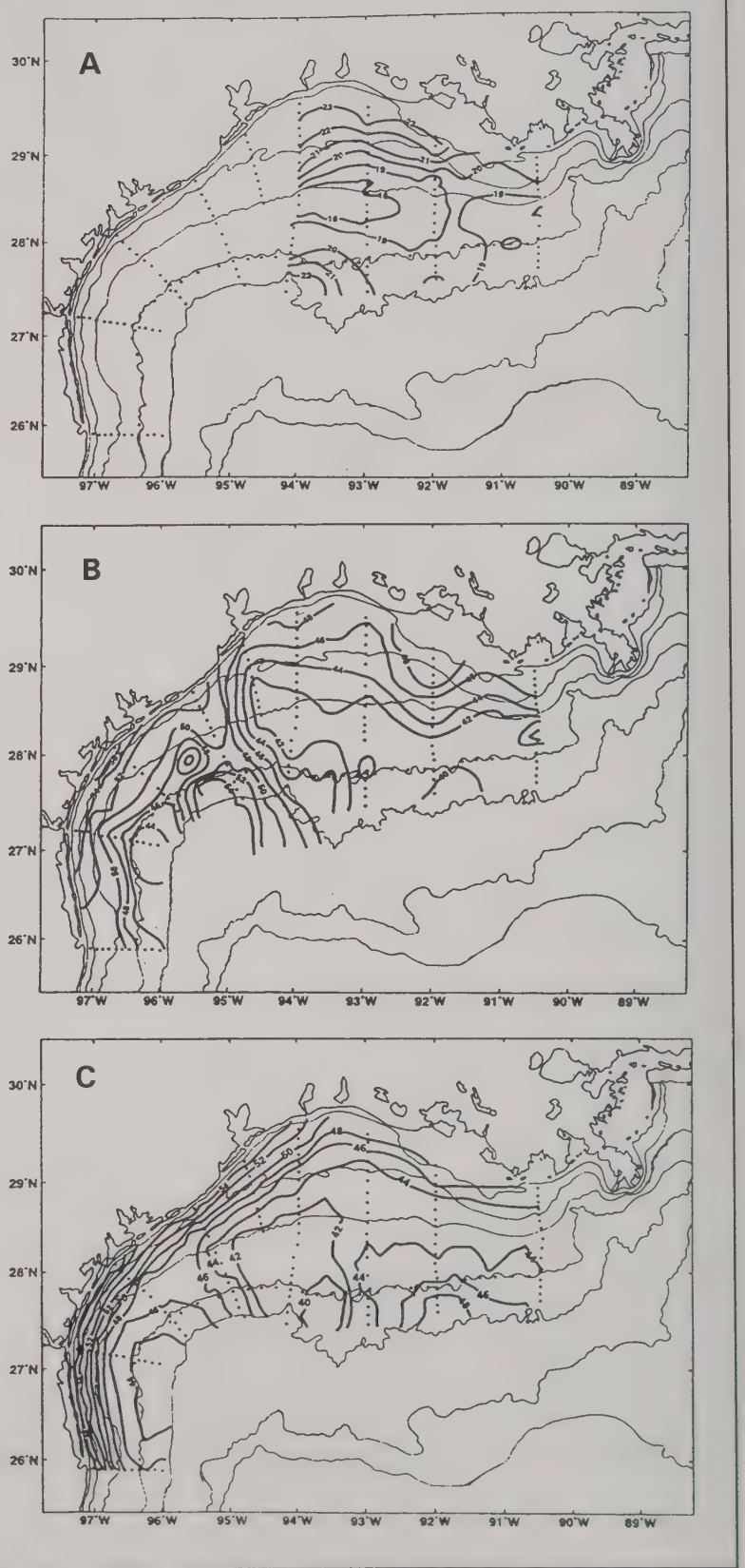
The Mississippi and Atchafalaya rivers introduce large volumes of fresh water onto the Texas-Louisiana shelf. Combined outflow from these two rivers accounts for up to 94% of the fresh water flow onto the shelf (Dinnel and Wiseman, 1986), and spring is the time of highest river flow. All other riverine input for the Texas-Louisiana shelf (15 rivers) is significantly lower than the Mississippi-Atchafalaya river system (Nowlin[2]). These minor inputs are not likely to have an effect on shelf hydrography in localized areas near the outflows, such as the Sabine River. River discharge influences hydrographic parameters such as salinity and nutrients, and the phytoplankton community can respond to these hydrographic changes within the water column (Riley, 1937; Edmond et al., 1981; Malone et al., 1983; Fransz and Verhagen, 1985; Xiuren et al., 1988; Lohrenz et al., 1990; Bidigare et al., 1993; Neuhard, 1994; Bontempi, 1995).

A cyclonic gyre circulation pattern prevails over most of the year on the Texas-Louisiana shelf. This pattern occurs throughout most of the winter and spring, breaking down in the summer months due to shifts in wind direction (Cochrane and Kelly, 1986). This circulation pattern has been confirmed with LATEX hydrographic data collected during seasonal cruises (Fig. 2). Geopotential anomaly contours clearly show the general circulation stated by Cochrane and Kelly (1986) for each of the spring cruises, May 1992 (Fig. 2A), May 1993 (Fig. 2B), and May 1994 (Fig. 2C). Downcoast flow

[1] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

[2] Nowlin, W. 1994. Department of Oceanography, Texas A&M University, College Station, TX 77843-3136.

Figure 2
Geopotential anomaly contours for the (A) May 1992 (92A-3db/70db), (B) May 1993 (93E-3db/200db), and (C) May 1994 (94H-3db/200db) LATEX hydrography cruises.



was observed on the inner shelf for all years, as was the cyclonic gyre circulation. Downcoast flow distributes lower salinity river water and associated (higher) nutrient levels across the Texas-Louisiana shelf.

Salinity

Figure 3A shows a vertical profile of salinity from spring 1992. The freshest water (S = 26.5) was found on the inner part of the shelf, and extended from near shore out to the 40 m isobath (Fig. 3A). In spring 1993, the freshest water was also found on the inner shelf within the study area (Fig. 3B), and was even less saline than in 1992 (S = 20.5). Low salinity waters on the inner shelf during 1993 are most likely due to record high flows from the Mississippi and Atchafalaya rivers for that year (Army Corps of Engineers[3]) (Fig. 4). The freshwater lens extended further seaward in May 1993 than in May 1992 as a result of the increased volume of river water flowing onto the shelf, which was also directed westward by the downcoast flow.

In the spring of 1994, the lowest salinity waters were observed at the inner shelf again (Fig. 3C). A surface salinity of 14.5 reflected the greater volume of fresh water from the Mississippi and Atchafalaya rivers in the first four months of 1994 than during previous years (U.S. Army Corps of Engineers[4]). Again, the influence of the predominant downcoast circulation in May 1994 was reflected in the surface salinity, indicating fresh river water located in a narrow band along the coast (Fig. 5). Possible localized riverine influences as evidenced by low salinity waters (Sabine River), are present on the innermost shelf area near the Texas coast along 94°W longitude.

Dissolved Oxygen

Hypoxia can result from a coupling of biological and physical processes on the Texas-Louisiana continental shelf (Rabalais et al., 1991). Texas-Louisiana hypoxia development is contingent upon the flow and freshwater volume of the Mississippi River, and is defined as the presence of water having a dissolved oxygen concentration below 1.4 $ml \cdot L^{-1}$ (Rabalais et al., 1991). Hypoxic conditions occur mainly in bottom waters, and are often found on the Louisiana continental shelf during the summer and can persist through October (Rabalais et al., 1991). These conditions may develop in the spring, but are generally confined to a limited area at that time. Fresh water on the shelf is warmed by increasing temperatures, creating a stable water column due to density differences in the water masses. Resultant stratification inhibits transport of oxygen to the lower layers of the water column, making the setting prime for hypoxic conditions to develop. Phytoplankton organic material from the spring bloom can sink, fueling respiration processes in both the water column and benthic environment. Respiration can utilize any available oxygen (Rabalais et al., 1991) and cause hypoxia to occur. Increased freshwater volume during the spring flood coupled with spring phytoplankton blooms can create conditions conducive to hypoxia. However, there were no bloom concentrations of any phytoplankton species detected in 1994 near the mortality area (LATEX A station 071).

The highest levels of dissolved oxygen in the three years of the study period were located on the inner shelf of Line 4 during May 1992 (Fig. 6A). On this transect, dissolved oxygen concentrations near the coast were 7.5 $ml \cdot L^{-1}$, and levels decreased along the bottom of Line 4 and from the inner to middle shelf. Vertical salinity contours indicated very little stratification on Line 4 during May 1992 (Fig. 6A), and no evidence of hypoxia was present on Line 4 at that time.

Dissolved oxygen values for May 1993 (Fig. 6B) were lower overall when compared with May 1992, and the amount of fresh water was greater during 1993. The highest concentration of dissolved oxygen in May 1993 was 5.5 $ml \cdot L^{-1}$, 2 $ml \cdot L^{-1}$ lower than in May 1992. Dissolved oxygen values ranged from 5.5 $ml \cdot L^{-1}$ at the surface to 4.5 $ml \cdot L^{-1}$ near the bottom during May 1993. Some stratification was present at that time, however, no evidence of hypoxic or anoxic events was found on Line 4 during spring 1993.

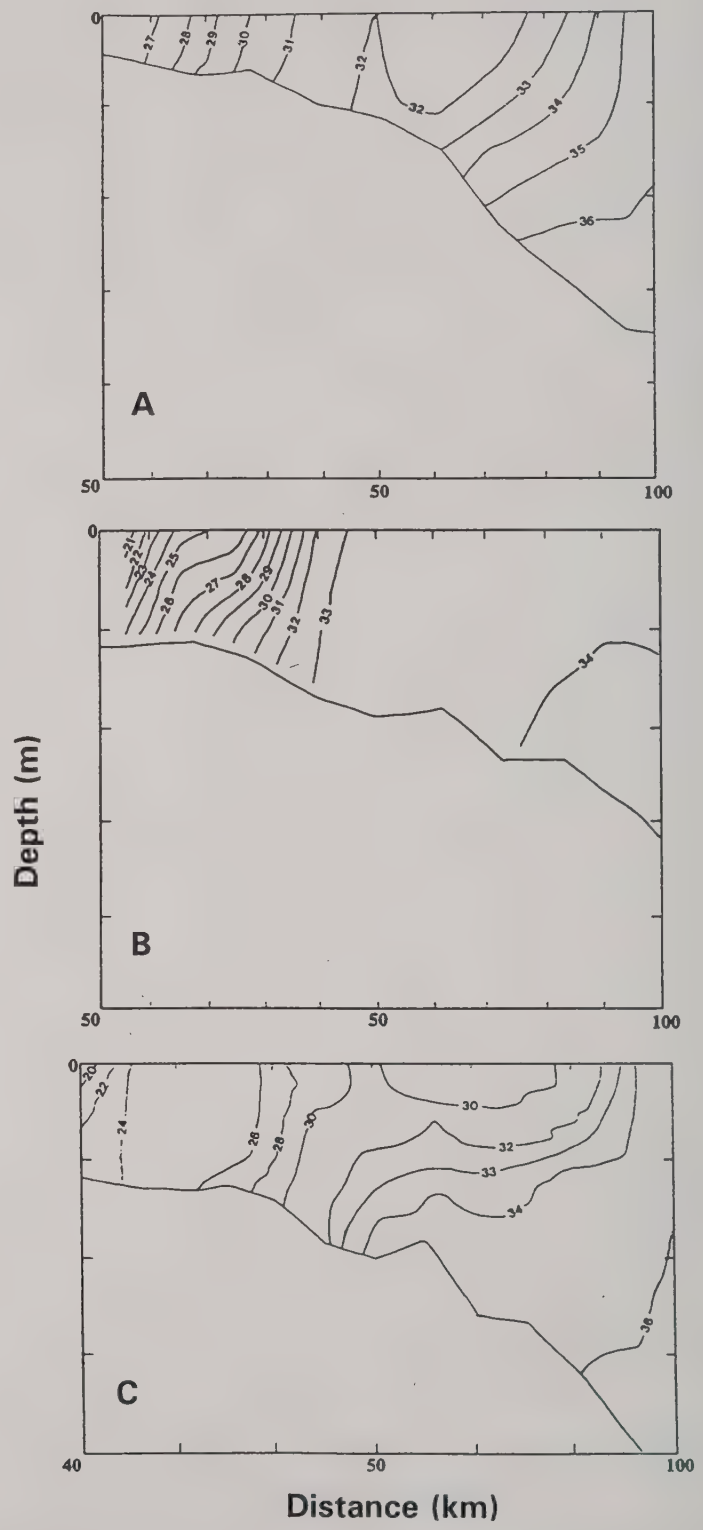
The May 1994 Line 4 dissolved oxygen contour (Fig. 6C) shows only the seven LATEX A stations closest to shore near the area of the mortality incident. Dissolved oxygen concentrations at these stations were the lowest of the 3 study years across the inner and middle shelf where the freshest water resided. Dissolved oxygen values on the innermost part of the shelf during May 1994 were 3.5–4 $ml \cdot L^{-1}$ lower than dissolved oxygen levels in 1992 and 1993, but were not indicative of hypoxic or anoxic bottom waters. Very weak stratification was present in May 1994 on Line 4 when compared to May 1993.

The water column near Sabine Pass, Texas, was mixed in 1992, and had less fresh water and higher dissolved oxygen levels present. In 1994, the lowest levels of dissolved oxygen were present when the water column was weakly stratified. No hypoxia was detected on Line 4 in the three years of springtime data.

[3] U.S. Army Corps of Engineers. 1993. Mississippi River flow database. New Orleans District, U.S. Army Corps of Engineers, P.O. Box 60267, New Orleans, LA 70160-0267.

[4] U.S. Army Corps of Engineers. 1994. Mississippi River flow database. New Orleans District, U.S. Army Corps of Engineers, P.O. Box 60267, New Orleans, LA 70160-0267.

Figure 3
Salinity contours of Line 4 for the (A) May 1992, (B) May 1993, and (C) May 1994 LATEX hydrography cruises.



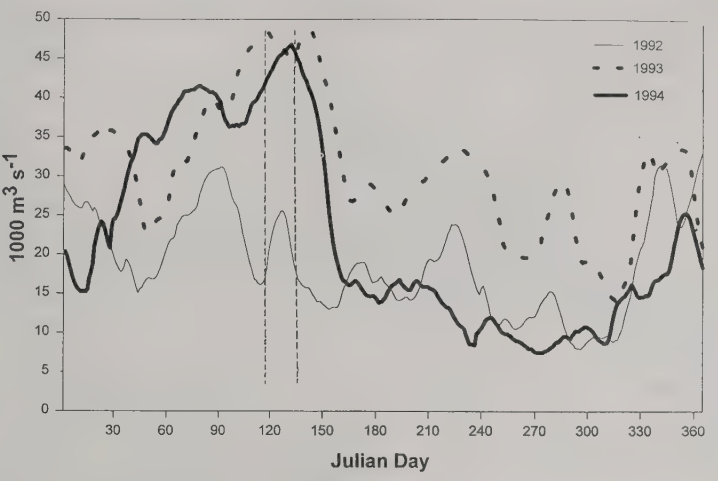


Figure 4
Mississippi-Atchafalaya river flow (1000 $m^3 \cdot s^{-1}$) versus Julian Day for the years 1992–94. The bars indicate the beginning and end of the cruise period.

Phytoplankton

Several species of phytoplankton are known to be toxic, but approximately 60 species of dinoflagellates are known to cause red tides (Texas A&M Univ. Sea Grant, 1986). About 30 of these dinoflagellate species can produce a toxin which could cause a major fish mortality event as occurred in 1994. Two toxic dinoflagellates common to Texas waters and known to cause red tides are *Gymnodinium brevis* (*G. breve*) and *Gonyaulax monilata* (*Alexandrium monilata*).

Phytoplankton samples were examined from springtime 1992 to 1994 near the area of the fish kill in an attempt to identify the presence of the aforementioned or any toxic dinoflagellate or diatom species, any dominant or bloom concentrations of phytoplankton, and phytoplankton species typically present during spring near Sabine Pass. Toxic phytoplankton could be a direct cause of the mortality event, while bloom concentrations of phytoplankton were speculated by Harper and Guillen (1989) to facilitate development of anoxic conditions and hydrogen sulfide production. Establishing the resident phytoplankton species and their abundances in the study area during spring may dispel any suspicion that a typically resident species may have caused the mortality incidents. For the three study years, phytoplankton abundances were highest within inner Texas-Louisiana shelf waters adjacent to the coast and river outflows. Greatest abundances were found in the springtime due to increased river flows and associated nutrient loads (Bontempi, 1995).

Line 4 (94°W) surface phytoplankton distributions from May 1992 are shown in Fig. 7. Inner and middle shelf abundances have been divided by 200 to enable a view of offshore group distributions. In May 1992, the abundance of phytoplankton was highest on the inner shelf, and diatoms were dominant. Dominant diatom species included a *Cerataulina* species, cf. *bergonii (pelagica)*, *Guinardia flaccida*, and several *Thalassiosira* species. Dominant dinoflagellates included species of the family Gymnodiniaceae (the majority under 20 μm in length) and some *Prorocentrum* species. Cryptomonads composed 47% of the microflagellates. These genera and species are common to the inner shelf on Line 4 during 1992. No known toxic algal species were present in May 1992 along Line 4 (94°W).

In May 1993, the highest surface abundance of phytoplankton was found on the inner shelf (Fig. 8), as observed in May 1992. Diatoms again composed the majority of the phytoplankton on the inner shelf. Dominant diatoms included *Skeletonema costatum* and a *Thalassiosira* species, and *S. costatum* was the most dominant diatom at the innermost station along 94°W in 1993. This cosmopolitan species is found to inhabit neritic waters of the world in a range of salinities and temperatures (Winsborough and Ward[5]; Malone et al., 1983; Marshall and Cohn, 1987; Xiuren et al., 1988; Medlin et

[5] Winsborough, B. M., and G. H. Ward. 1979. Phytoplankton distribution and community structure in Trinity Bay, Texas. Submitted by Espey, Huston and Associates, Inc. Presented at First Winter Meeting, American Society of Limnology and Oceanography, Corpus Christi, Texas.

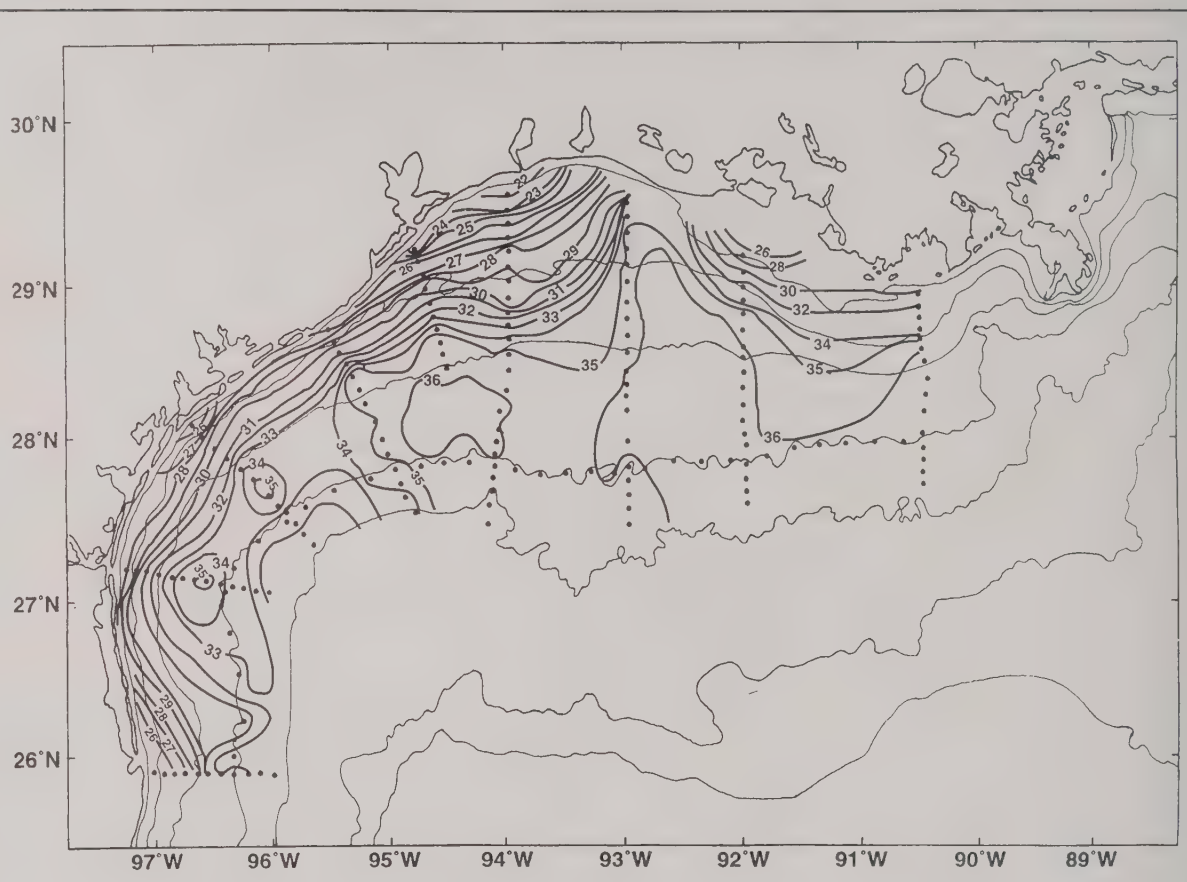


Figure 5
Surface salinity contour for the May 1994 (94H) LATEX hydrography cruise. Note the location of the fresher Mississippi, Atchafalaya, and Sabine river water along the coast.

al., 1991) and colonizes well in culture on different media (Villac[6]). Colonization of the inner shelf area by *S. costatum* may be a consequence of increased river flow and resultant change in the shelf environment (salinity, nutrients, etc.). In 1993, Gymnodiniaceae were the majority of the dinoflagellates, and cryptomonads composed the majority of microflagellates, as in 1992. No known toxic algal species were present on Line 4 in May 1993.

Diatoms dominated the phytoplankton population at station 071 in May 1994 (Fig. 9). Dominant diatoms included three species of Chaetoceroceae and *S. costatum*. Again, increased freshwater volume on the shelf probably created an environment in which a cosmopolitan diatom species, *S. costatum*, could proliferate. Three species of cryptomonads composed 94% of the microflagellates. Gymnodiniaceae and a *Protoperidinium* species were dominant dinoflagellates. No toxic algae were found to be present at the station nearest the mortality site during May 1994.

Abundance numbers of phytoplankton were highest on the inner shelf along Line 4 in 1992 and 1993, and most likely during 1994 as well. No bloom concentrations of a single phytoplankton species were identified, however. Cryptomonads contributed significantly to the Line 4 inner shelf microflagellate population during the spring period, and Gymnodiniaceae were common dinoflagellates. No known toxic algal species of diatoms or dinoflagellates were identified on the inner shelf along 94°W during May 1992, 1993, or 1994.

Percent Transmission and Wind

Water samples examined from May 1994 were very heavily sedimented and an 80% dilution was necessary

[6] Villac, C. 1994. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. Personal commun.

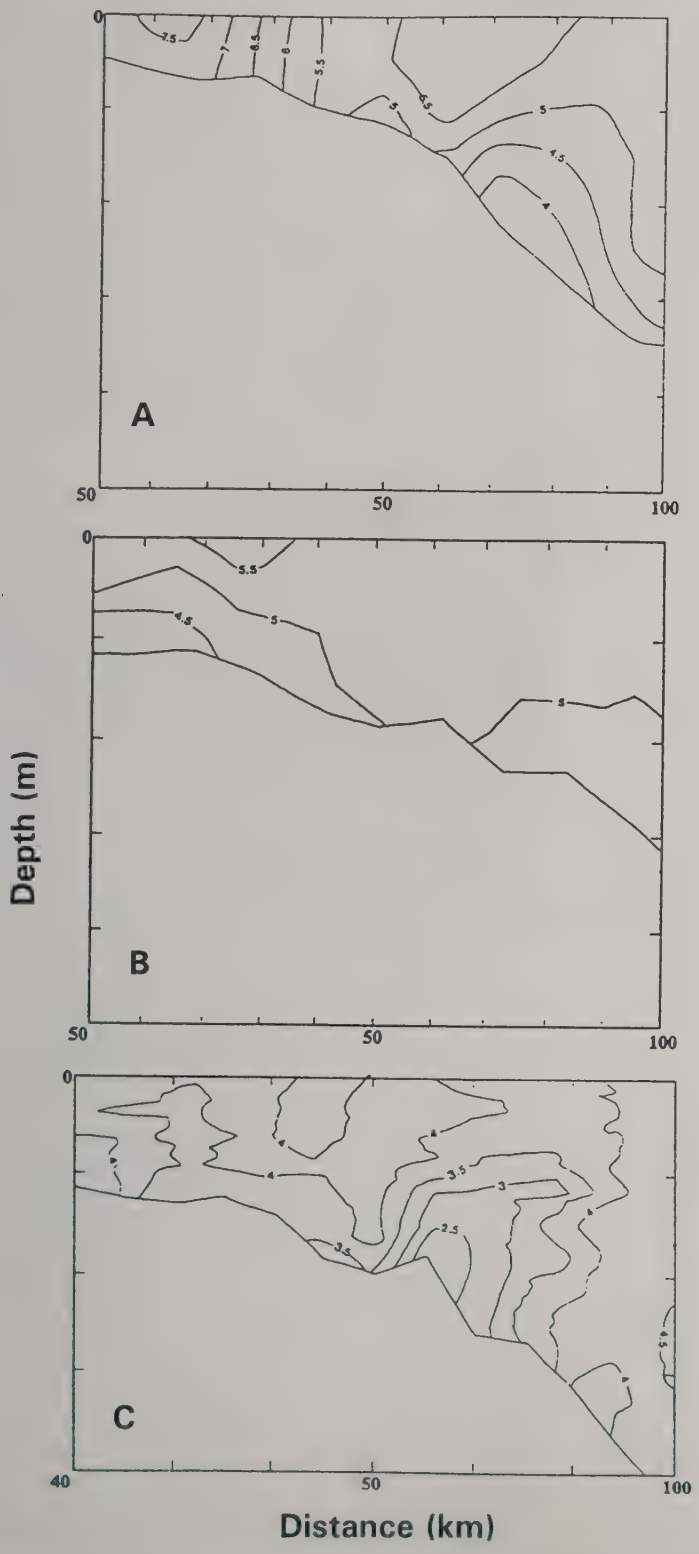


Figure 6
Dissolved oxygen contours (ml·L^{-1}) of Line 4 for the (A) May 1992, (B) May 1993, and (C) May 1994 LATEX hydrography cruises.

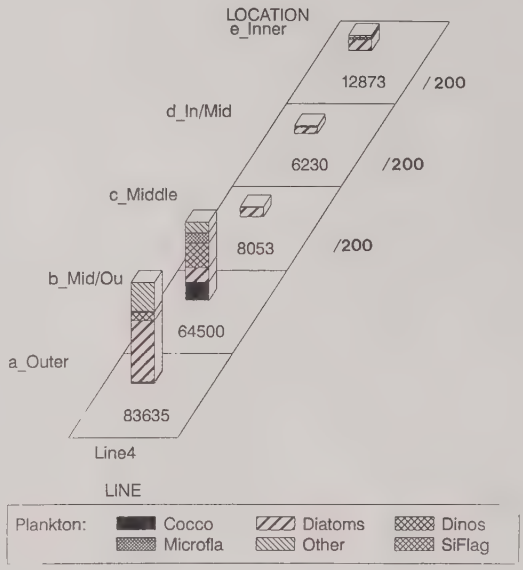


Figure 7
Phytoplankton abundance (cells·L^{-1}) and group distributions at the surface of the Texas-Louisiana continental shelf in May 1992. View looking from the outer shelf towards the shore, with the inner to the middle shelf abundances divided by 200 to enable a view of outer shelf phytoplankton groups.

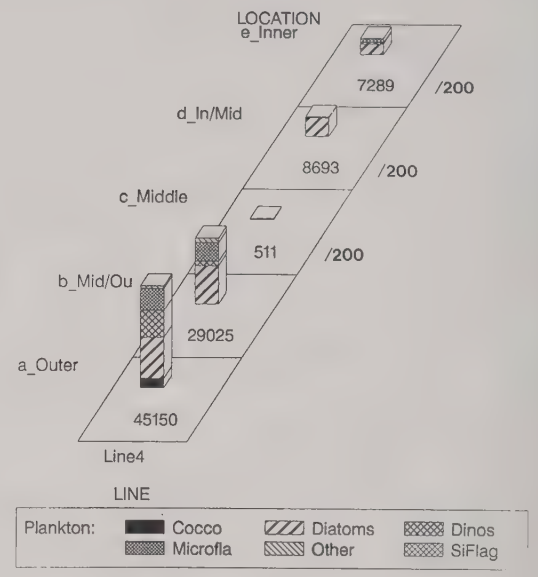


Figure 8
Phytoplankton abundance (cells·L^{-1}) and group distributions at the surface of the Texas-Louisiana continental shelf in May 1993. View looking from the outer shelf towards the shore, with the inner to the middle shelf abundances divided by 200 to enable a view of outer shelf phytoplankton groups.

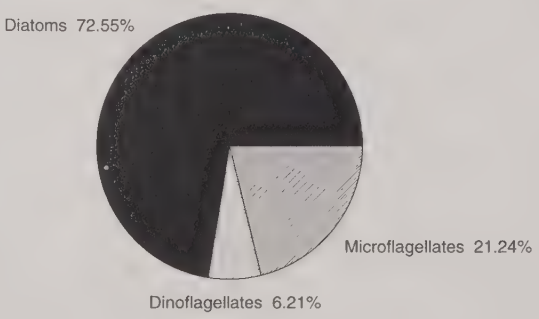


Figure 9
Percent composition of phytoplankton groups (diatoms, microflagellates, dinoflagellates) near the mortality event at station 071 on Line 4 in May 1994.

to enable identification of phytoplankton from that cruise. Heavy sedimentation was reflected in the percent light transmission contour for Line 4 during May 1994 (Fig. 10). On the inner shelf around station 071, light transmission was about 20%. In this area on Line 4 in May 1992 and 1993, the percent transmission was 36% and 47%, respectively. High turbidity may have been occurring in the water column during May 1994. Turbidity may have resulted from mixing due to increased Mississippi River flow in 1994, and could be reflected in the lack of stratification on the inner shelf as shown in the May 1994 vertical salinity contour (Fig. 3).

During the period from 4 April to 11 May 1994, mean monthly wind direction was from the south and southeast (Howard[7]). During SE-SSE winds, current flow is downcoast and surface waters move shoreward in an Ekman layer (Howard[7]). There were a few very short wind events (maximum 24 hour duration) occurring on or around 15 April, 18 April, 25 April, 2–3 May, and 5 May when winds were from a north/northeasterly direction.

Conclusions

We have not identified a cause for marine mortality events near the Sabine River in May and June 1994

[7] Howard, M. 1993. Department of Oceanography, Texas A&M University, College Station, TX 77843-3146. Personal commun.

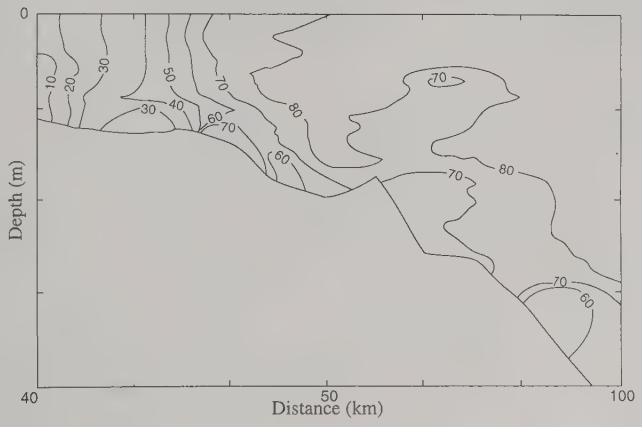


Figure 10
Percent transmission contours for Line 4 during the May 1994 (94H) LATEX hydrography cruise.

based on the LATEX A and ONR data reported here. However, we can conclude that in 1994, seaward of the fish kill area, the water column was not strongly stratified and no hypoxia, toxic phytoplankton species, or bloom concentrations of phytoplankton were detected. Oxygen levels were too high for hydrogen sulfide to form in the LATEX A study area during 1994. Further examination of data from within the actual mortality incident area, including dissolved oxygen, phytoplankton abundance, and species composition, and wind and current data may help identify probable causes.

Some phytoplankton may be characteristic of the inner Texas-Louisiana shelf area, including chain-forming diatoms, *Leptocylindrus danicus* and *Skeletonema costatum*, dinoflagellates of the family Gymnodiniaceae, and several species of cryptomonads. The increased freshwater volume in 1993 and 1994 may be identifiable by the dominance of *S. costatum*.

Station 071 in May 1994 was about 6–7 km from the initial mortality incident area, so data presented here may provide some insight into features and processes occurring on the Texas-Louisiana shelf seaward of the 10-m isobath under different flow regimes of the Mississippi River. Studies conducted within the actual mortality incident area by the National Marine Fisheries Service and the Texas Parks and Wildlife Department may identify a direct cause of the mortality events.

Acknowledgments

Thanks to Ann Jochens, the LATEX Deputy Program Manager at Texas A&M University for her initial insights on this work, and Yongxiang Li at Texas A&M University for the dissolved oxygen, salinity, and geopotential anomaly contours. Thank you to Denis A. Wiesenburg and Kelly R. Thornton at the University of Southern Mississippi's Center for Marine Sciences for their insight, review, and editorial work; Matt Howard at Texas A&M University for the wind information; and to Greta A. Fryxell at Texas A&M University for her opinions regarding the toxic phytoplankton and red tide information. This work was funded by the Minerals Management Service LATEX program (OCS contract number 14-35-0001-30509) and the Office of Naval Research contract number N00014-93-1-05130.

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Phytoplankton Blooms off Louisiana and Texas, May–June 1994

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ABSTRACT

Phytoplankton blooms were coincident with mass mortalities of fish and crustaceans along the Louisiana and northeast Texas coasts in May and June 1994. Phytoplankton communities in water samples collected from fish kill and adjacent areas were composed of mixed diatom, dinoflagellate, and microflagellate species common to Gulf of Mexico coastal waters. However, two species clearly dominated; both species have previously been associated with fish kills. *Heterosigma* cf. *akashiwo* dominated Louisiana samples and *Gymnodinium sanguineum* dominated Texas samples. Both species can be noxious and are potentially toxic. Although the cause(s) of this marine mortality event has not been determined, one likely scenario involves the direct or indirect effects of phytoplankton blooms.

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Introduction

Unusually high mortalities of dolphins and sea turtles occurred on the Texas coast between January and May 1994, followed by mass mortalities of fishes and crustaceans along the upper Texas coast to Louisiana. Coincident with mass mortalities in May and June, tea-colored patches of water were observed near- and offshore. A phytoplankton bloom was suspected as the cause of sea-surface discoloration as well as the mortalities of black drum, *Pogonias cromis*, pompano, *Trachinotus carolinius*, blue crabs, *Callinectes* spp., and bottom-dwelling fishes, including hardhead, *Arius felis*, and gafftopsail catfish, *Bagre marinus*. Catfish were the most abundant dead fish stranded on beaches and showed none of the abrasions and marks associated with fish that have been discarded from net hauls; fishing activity was therefore ruled out as a cause.

Methods and Materials

Surface samples were collected 9 May and 12 May from nearshore waters off Cameron, Louisiana (live, Lugol's fixative, and/or glutaraldehyde); 12 May from discolored water in Rollover Pass, Texas; 16 May (Lugol's) off Sabine Pass and Galveston; 3 June (live and Lugol's) from discolored water and clear water off Sabine Pass; and 22 June and 24 June (live, Lugol's, and gluteraldehyde) from discolored water, boundary water, and clear water off Sabine Pass. Salinities of these water samples ranged from 12–33‰; inshore samples had the lowest salinities. Standard light microscopy techniques, e.g., bright field, phase contrast, differential interference, and epifluorescence optics, were used to identify the dominant phytoplankton. Numerical abundance of the dominant species was estimated by counting whole l-ml or 0.1-ml aliquots of live or preserved material in duplicate, when possible. Scanning electron microscopy techniques followed the method of Steidinger et al. (1989), which is a simultaneous GTA-OsO_4 at 4°C where the osmolality of the fixative and buffer is adjusted to the osmolality of the sample. Critical-point-dried and coated material was observed with a Leica[1] Stereoscan 240 SEM.

Most of the phytoplankton taxa identified are illustrated in Steidinger and Williams (1970), Fukuyo et al. (1990), and Steidinger (1996).

Results

The 12 May sample collected off Cameron had a mixed phytoplankton community characteristic of brackish or estuarine water. One of the dominant flagellates was *Heterosigma* cf. *akashiwo* at cell counts of 3×10^6 L^{-1}. In addition to this raphidophyte, a mixed community of diatoms, dinoflagellates, and microflagellates was present. Many of the species were in the smaller size class of <20 μm. There were small centric diatoms such as *Thalassiosira*, euglenoids, chloromonads, and the following dinoflagellates: *Katodinium glaucum*, *Heterocapsa rotundata* (=*Katodinium rotundatum*), *H.* cf. *pygmaea*, *Heterocapsa* sp., *Woloszynskia* spp., *Scrippsiella* sp., *Oblea* sp., *Protoperidinium* sp., *Gyrodinium* spp. (heterotrophs), *Gymnodinium* spp. (heterotrophs), and a new peridinioid species. Most of the small peridinioid cells (8 to 15 μm) appeared to be unarmored gymnodinioid forms at the light microscope level of resolution, but scanning electron microscopy showed them to be either armored or of the *Woloszynskia* type (see Steidinger et al. 1996b). Many of the dinoflagellates lacked chloroplasts and were considered heterotrophic. The 12 May Rollover Pass sample was dominated by *Gymnodinium sanguineum* (=*G. splendens*, *G. nelsonii*) at 1.9×10^5 cells L^{-1}; *H.* cf. *akashiwo* was present but not abundant.

Samples collected 16 May off Sabine Pass and Galveston Bay had no dominant phytoplankton bloom organism.

Three samples collected 3 June off Sabine Pass contained *Gymnodinium sanguineum* (13×10^4 to 1.23×10^6 cells L^{-1}), *Dinophysis caudata* (5 to 8×10^3 cells L^{-1}), *Prorocentrum* cf. *compressum*, *P. gracile*, *Katodinium glaucum*, *Scrippsiella* cf. *trochoidea*, *Lingulodinium polyedra*, *Gonyaulax polygramma*, *Ceratium hircus*, *C. fusus*, *Torodinium robustum*, *Pseudosolenia* (=*Rhizosolenia*) *calcar-avis*, *Proboscia* (=*Rhizosolenia*) *alata*, *Rhizosolenia delicatula*, *Cyclotella* spp., *Thalassiosira* spp., *Chaetoceros* spp., and other phytoplankters. The sample from the discolored water had the highest concentration of phytoplankton.

Samples collected 22 June west of Sabine Pass, Texas, were from (1) a discolored water patch, (2) at the edge of the discolored water, and (3) in clear water. Both the discolored and edge samples had similar species composition, but the edge sample had less dense populations. The most abundant species were *Gymnodinium sanguineum* (5.8×10^4 to 5.5×10^6 cells L^{-1}), *Prorocentrum minimum* (var. *triangulatum*) (up to 1×10^5 cells L^{-1}), *P. minimum* (var. *minimum*), *P. compressum*, *P. micans*, *Ceratium hircus*, *Scrippsiella trochoidea*, *Heterocapsa rotundata*, *H.* cf. *niei*, *Lingulodinium polyedra*, two *Eutreptiella* spp. (euglenoids) (up to 4.3×10^6 cells L^{-1}), small peridinioid dinoflagellates (up to 5×10^5 cells L^{-1}), *Dictyocha* sp. (silicoflagellate), and tintinnids. Water samples collected 24 June contained resuspended sediments, pennate diatoms, centric diatom frustules, fecal pellets, pollen, sand, euglenoids, dinoflagellate cysts and cyanobacteria but no planktonic bloom.

[1] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Discussion

Louisiana Samples

Heterosigma cf. *akashiwo* was the dominant phytoplankter in the 12 May Cameron, Louisiana, sample. A bloom of *H.* cf. *akashiwo* was also observed in estuarine and coastal waters off Terrebonne Bay, Louisiana, in March 1994 (Dortch and Robichaux[2]). The species can be associated with areas in which freshwater runoff is high and levels of dissolved oxygen are low (Honjo, 1992). In certain areas and evidently under varied environmental conditions, *H. akashiwo* can cause fish kills. At certain growth phases, a Japanese isolate produces hydrogen peroxide and bioactive superoxide radicals that can kill fish under experimental conditions (Yang et al., 1993). A geographic isolate of *H.* cf. *akashiwo* from Louisiana coastal waters (or sediments) should be cultured to determine its potential toxicity to fish and to further characterize its physiological tolérances and requirements in relation to growth and life history. This species produces bottom-resting stages (Tomas, 1978) that may be significant as a seed source. Resting stages were found in a May 1994 Cameron water sample.

The Cameron sample also contained small (8 to 20 μm) dinoflagellates that were lightly armored; some of them are new species. Scanning electron microscopy had to be employed for the Cameron sample to rule out the presence of the phantom-type dinoflagellate, *Pfiesteria piscicida*, recently found in North Carolina and east coast estuarine waters, that causes fish kills (Burkholder et al., 1992; Steidinger et al., 1996a). When fish kills are associated with high concentrations of small "gymnodinioid"-appearing dinoflagellates, particularly those <15 μm, scanning electron microscopy is the only tool currently available for positive identification. In the future, various molecular probes may be used to identify harmful or toxic species biochemically, e.g., through cell-surface recognition with immunoassays.

Texas Samples

Samples collected in discolored-water patches off Texas were dominated by the small- to medium-sized unarmored dinoflagellate *Gymnodinium sanguineum*. This species has previously caused discolored-surface-water events worldwide and has even been associated with mass marine mortalities. Most of the fish kills were attributed to respiratory failure brought on by physical impairment of the gills or by low levels of dissolved oxygen in the seawater that were caused by the dinoflagellate bloom. This species is thought to be nontoxic and has even been used successfully as larval fish food (Scura and Jerde, 1977). However, Tindall et al. (1984) found that extracts of a Caribbean isolate of *G. sanguineum* were toxic to mice, and *G. sanguineum* has been implicated in mortalities of oyster larvae and juveniles (Cardwell et al., 1979; Bricelj et al., 1992). Algal extracts from water samples collected from discolored-water patches off Texas were non-toxic when assessed by mouse bioassay and cytotoxicity assays but displayed PbTx-like activity in a receptor assay (see Van Dolah et al., 1998).

Prorocentrum minimum and *Dinophysis caudata* are recognized toxic dinoflagellate species associated with shellfish poisonings. In addition, *P. minimum* has been associated with fish kills off Florida's west coast, but the cause was thought to be anoxic conditions (Smith, 1976). Both of these species co-occurred with *G. sanguineum* in some samples. Another toxic dinoflagellate, *G. mikimotoi* (=*G. nagasakiense*, *G.* cf. *aureolum*), was tentatively identified in initial live samples but was not verified in preserved samples nor in subsequent live and preserved samples. It has been recorded from the Gulf since the 1960's (see Steidinger and Williams, 1970, *Gymnodinium* E) and can reach bloom concentrations.

Gymnodinium sanguineum is so variable in size and shape that it can be difficult to identify because some of the morphs may appear to be different species. In log-growth concentrations, *G. sanguineum* typically has a very characteristic shape with a dorsoventrally flattened cell that has a broad conical or semihemispherical epitheca (=epicone), a bilobed hypotheca, a median slightly displaced cingulum, and a characteristic cytology. In bloom concentrations and in stationary growth of cultures, *G. sanguineum* is extremely pleomorphic, ranging from circular in cross section to lightly pigmented to lacking the bilobed hypotheca although there may be an indentation (see Steidinger and Williams, 1970). It may be that some of these forms represent life-cycle stages or variants induced by different salinities or by growth phase. Although the position of the nucleus and the shape and positioning of chloroplasts can vary with age and environmental conditions (e.g., turbulence), they often help in identifying morphs within a bloom. In one sample at the edge of a bloom (22 June), cells were enclosed by a prominent mucoid halo, and again, this condition could have represented a life-cycle stage (e.g., a resting stage). Voltolina (1993) attributed recurring blooms of *G. sanguineum* in a British Columbia lagoon to overwintering cysts, or resting stages, of an undescribed nature.

Gymnodinium sanguineum has previously bloomed along the Texas coast under similar environmental con-

[2] Dortch, Q. (Louisiana Universities Marine Consortium, 8124 Highway 56, Chauvin LA 70344), and R. J. Robichaux (Louisiana Department of Environmental Quality, Bayou Regional Office, 104 Lococo Dr., Raceland, LA 70394). Unpubl. data.

ditions. Harper and Guillen (1989) reported on a *G. sanguineum* (as *G. splendens*) bloom in June 1984 in low-salinity water from Galveston, Texas, to Cameron, Louisiana. The bloom was also associated with mortalities of fishes and crabs along that coastline. The mass mortalities were preceded by heavy runoff from the Mississippi-Atchafalaya rivers, along-shore downcurrent transport induced by winds, and the appearance of a *G. sanguineum* bloom. These authors attributed the kills to hypoxia and/or hydrogen sulfide resulting from the bloom. Although the fish species affected in the 1984 and 1994 bloom events differed, most of them would be susceptible to hypoxic conditions. Hypoxia along the northern Gulf of Mexico shelf is a documented event. Shelfwide surveys in April of 1992 and 1993 and July of 1985–1994 show that bottom water oxygen concentrations of <2 mg L^{-1} do not extend as far west as Calcasieu in the spring and usually not in the summer (Rabalais et al., 1991; Rabalais et al.[3]). While this argues against annual, shelfwide hypoxia (Rabalais et al., 1991) as a cause, it does not rule out a small-scale hypoxic event specifically related to the bloom. In all likelihood, the causes of the 1984 and 1994 mass mortality events are very similar. Data about the toxicity of *G. sanguineum*, the distribution of oxygen during the blooms, and the mass mortalities are insufficient to determine a cause in either year. Because this is a recurrent phenomenon, a plan for future response should be prepared.

Acknowledgments

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Addendum

Since the submission of this manuscript, another phytoplankton bloom occurred in June 1996. A bloom sample from the Gulf of Mexico near Sabine Pass, Texas, had a similar *Gymnodinium sanguineum* bloom (1.5×10^6 cells L^{-1}) that discolored the sea surface. An adjacent nondiscolored water area had 3×10^3 *G. sanguineum* cells L^{-1}.

Occurrence of *Gymnodinium sanguineum* in Louisiana and Texas Coastal Waters, 1989–94

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ABSTRACT

An area of discolored water, caused by a bloom of *Gymnodinium sanguineum,* and an associated fish kill occurred on the upper Texas and western Louisiana coasts in June 1994 in the same area where a similar bloom and fish kill occurred in June 1984. Because of the recurring association between blooms of *G. sanguineum* and fish kills, the distribution and ecology of this dinoflagellate was examined. The purpose was to summarize what is now known about this organism and to suggest areas that require further research. Data collected in the Louisiana-Texas coastal zone from 1989 to 1994, both prior to and in response to this event, indicates that *G. sanguineum* was present in approximately 8% of all samples. It is most often found in the summer at low salinities in the plumes of the Mississippi and Atchafalaya rivers. By comparison with environmental conditions associated with *G. sanguineum* elsewhere, it is hypothesized that *G. sanguineum* prefers stable environments with high nutrient availability. Live samples from the bloom site, incubated for one month, produced cyst-like structures. Their role in seeding blooms cannot be assessed until more is known about the nature of the cysts and the overall life cycle of *G. sanguineum.* Finally, the relationship between *G. sanguineum* and fish kills cannot be determined until the possible toxicity of *G. sanguineum* is investigated.

Introduction

A series of fish kills occurred from April to June 1994 along the upper Texas and western Louisiana coasts, sometimes associated with discolored waters (Denton et al., 1998). Finally, in late June, there was an extensive fish kill associated with "tea-colored" water. The fish mortalities, consisting primarily of demersal finfish, were believed to be linked to the bloom events. Steidinger et al. (1998) identified a suite of potentially toxic phytoplankton from samples taken over the course of these episodes, with the dinoflagellate *Gymnodinium sanguineum,* reaching bloom proportions in the "tea-colored" water in late June. Fish kills associated with algal blooms have been observed in the past on the upper Texas coast (Wilson and Ray, 1956; Wardle et al., 1975; Harper and Guillen, 1989), and *G. sanguineum* was also associated with one of these events in June 1984 (Harper and Guillen, 1989).

We report here on the temporal and spatial distribution of *G. sanguineum,* determined from sampling the Louisiana-Texas shelf and Louisiana estuaries from 1989 to 1994. In addition, we present evidence for the formation of a possible cyst by *G. sanguineum* in water samples from the bloom. Our purpose is to put the bloom and fish kill event into a larger regional context and to summarize what is now known about the growth habits

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of this organism in order to hypothesize the factors that may lead to blooms and fish kills.

Materials and Methods

Phytoplankton Surveys

Phytoplankton samples were collected and counted during 19 shelf-wide cruises from 1989 to 1993, spanning all months except Nov.– Feb. The sampling locations are given in Fig. 1, but not all stations were sampled on all cruises. Stations in the eastern area between the Mississippi and Atchafalaya rivers were sampled much more frequently than those to the west. From 1990 to 1993, a station in the coastal zone (28°52.18'N, 90°28.94'W) was sampled at least monthly except in the winter. Weekly sampling was conducted at three stations in the Terrebonne Bay estuary from 1993 to the present.

After the discolored water and fish kill occurred alongshore off Sabine Pass, samples were taken from a number of other Louisiana estuaries by us and by the Louisiana Department of Wildlife and Fisheries. The purpose was to determine the geographical distribution of the organism and especially to determine if bloom concentrations were occurring in nearby areas.

Bloom Samples

Surface samples were collected by the Texas Parks and Wildlife Department on 22 June 1994 from areas of discolored water near Sabine Pass and sent to Louisiana Universities Marine Consortium. One sample was collected for live cells and another was fixed with gluteraldehyde. Upon arrival, live samples were placed in an incubator on a 14/10 light/dark cycle at 28.5°C, to provide conditions as similar as possible to those experienced in the field. After one month of incubation, an aliquot of the live sample was preserved and the rest was split. One half was kept in the incubator as before; the other half was put in the dark at 22.6°C. Fixed samples were counted as described below.

Preservation and Counting

The fixed samples were preserved and counted according to a method adapted from Murphy and Haugen (1989) and Shapiro et al. (1989). Samples were preserved using 0.5% gluteraldehyde and kept refrigerated in the dark for at least one hour. Aliquots were stained with 0.03% proflavine hemisulfate, filtered through 8μm polycarbonate filters, and mounted on slides in low fluorescence immersion oil. Phytoplankton were then counted using an Olympus[1] epifluorescence microscope with blue, green, and transmitted light.

Palynological Processing

Preserved samples, both from the initial bloom sample and from subsequent incubations, were processed us-

[1] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

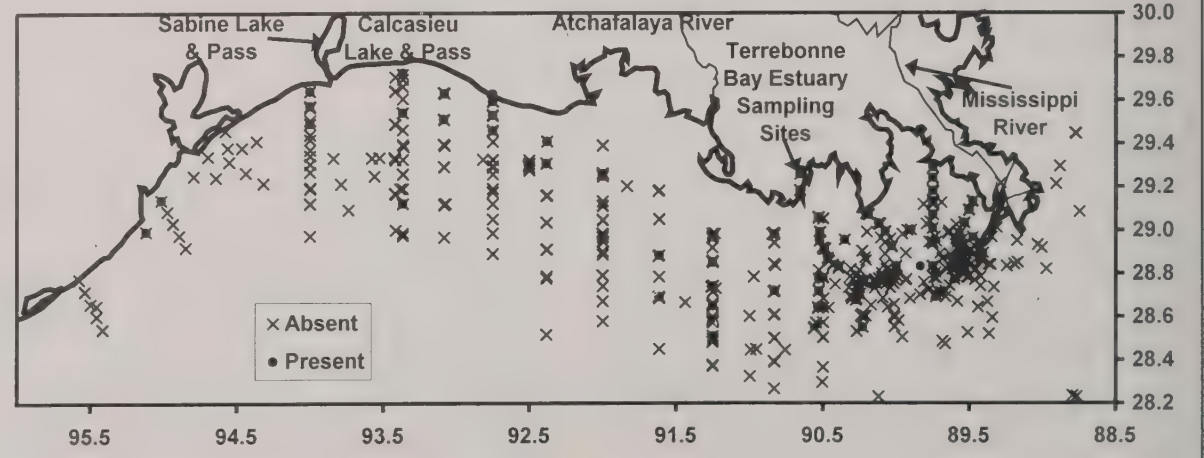

Figure 1
Stations sampled from 1989–93 at which *G. sanguineum* was present or absent.

ing standard palynological acid digestion procedures to extract and concentrate any acid resistant, organic-walled cysts that might be present. This consisted of treatment with cold 10% hydrochloric acid (HCl), cold concentrated hydrofluoric acid (HF, 51%), and hot concentrated HCl (38%). Residues were rinsed three times with distilled water after each digestion step. Permanent microscope slides were made of the residue following each digestion and examined with a light microscope.

Results

Cyst Formation in the Bloom Sample

The dominant phytoplankter in the bloom sample was identified by Steidinger et al. (1998) as *G. sanguineum* (= *G. splendens*), which was present at concentrations of 5.37×10^6 cells l^{-1}. However, morphology and cell size (Table 1) were quite variable. Only 1% of the cells were the characteristic dorsoventrally flattened cell with a bilobed hypotheca. The rest were round cells lacking any indentation of the hypotheca. Nucleus position and chloroplast arrangement were similar among the morphological variants. Steidinger et al. (1998) made similar observations from other samples taken at the bloom site. The difference in morphology was most evident when live cells were observed in motion. The smaller, round cells swam in a consistent helical path, whereas the larger, flattened cells swam in one plane, only rotating occasionally. Other dinoflagellates were also present in the sample, including *Prorocentrum micans* (1×10^3 cells l^{-1}), *P. compressum* (8×10^3 cells l^{-1}), *Ceratium hircus* (1×10^3 cells l^{-1}), and *Scrippsiella* sp. (1.8×10^5 cells l^{-1}).

After one month there were no vegetative cells in the live sample placed in an incubator in the light, but cyst-like structures (Fig. 2) were present at concentrations of 6.50×10^5 cysts l^{-1} (Table 1). All contained a typical dinoflagellate nucleus and a red accumulation body, similar to that observed in cysts of another naked dinoflagellate (Anderson et al., 1988). The sample was then split; one portion was incubated in the light as before and the other was incubated in the dark at a lower temperature. In general it is believed that cysts remain dormant in the dark at temperatures below the normal growth temperature (Pfiester and Anderson, 1987). One month later (Table 1), the sample in the light contained neither vegetative cells nor cyst-like structures. The sample in the dark at a lower temperature had cyst-like structures, but concentrations had decreased and the morphology had changed over the course of the month. Although they still retained the red accumulation body, the nucleus was no longer pronounced; they were rounder and not surrounded by an

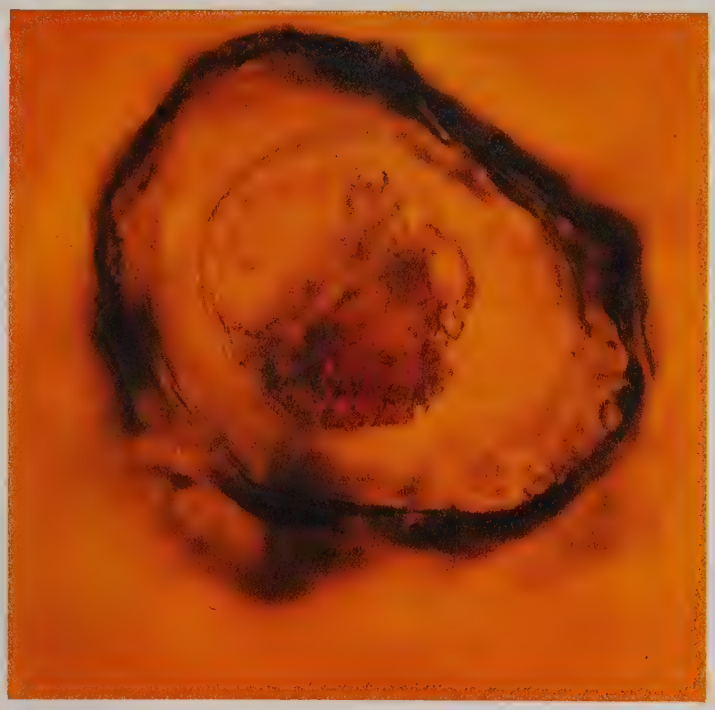

Figure 2
Single cyst–like structure showing red accumulation body (1000×). Dimensions of outer structure are 44 μm × 34 μm. Dimensions of internal structure are 30 μm × 18 μm.

Table 1
Numbers and sizes (length × width) of vegetative cells in original, preserved sample and cyst-like structures in the live sample incubated in the light at 28.5°C (22 June 1994 to 20 July 1994) and the dark at 22.6°C (20 July 1994 to 22 September 1994)

	Cells	Cysts	Cysts
Date	6/22/94	7/20/94	8/22/94
Concentration (cells l^{-1})	5.37×10^6	6.50×10^5	1.26×10^1
Maximum size (μm)	61 × 55	48 × 40	63 × 47
Minimum size (μm)	28 × 28	40 × 28	39 × 39

outer layer, and they contained one or more round, green-fluorescing bodies.

No cysts were observed at any stage in the palynological processing, indicating that cysts were not coated with acid-resistant material, such as sporopollenin. However, it was noted that *G. sanguineum* vegetative cells were resistant to all acid digestion except immersion in concentrated HCl. The flagella remained attached to the theca after digestion in cold 10% HCl. Since the flagella is attached to the outer membrane, that must also still be present after cold 10% HCl treatment. Minor degradation was noted on specimens subjected to HF digestion, mainly a partial detachment of the transverse flagella from the cingulum. But the theca appeared to be in overall good condition. Digestion in concentrated HCl, hot or cold, resulted in destruction of almost all vegetative cells of *G. sanguineum*. The rare specimens observed were nearly unrecognizable because the overall structure was degraded.

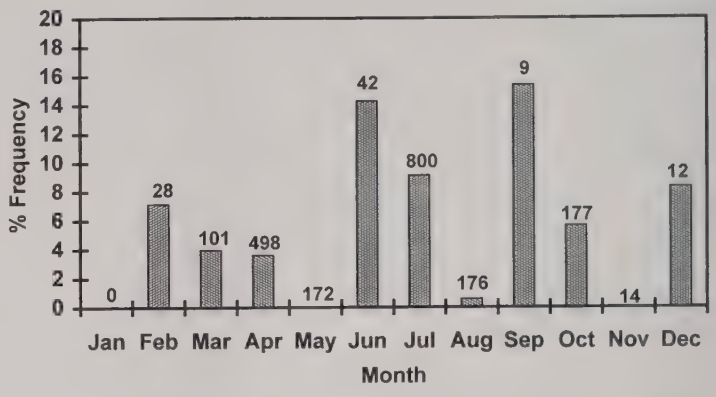

Figure 3
Percent frequency of *G. sanguineum* (number of samples with *G. sanguineum*/ total number of samples). Numbers above each bar indicate the number of samples for each month.

G. sanguineum Distribution in Louisiana Coastal Waters at the Time of the Bloom

At approximately the same time as the bloom off of Sabine Pass, high numbers of *G. sanguineum* (10^4–10^5 cells/liter) were observed in routine sampling at the lowest salinity station in the Terrebonne Bay estuary and at a much higher salinity just outside of Terrebonne Bay. Because of possible negative impacts of *G. sanguineum* on oysters and oyster larvae (Cardwell et al., 1979; Bricelj et al., 1992), more widespread estuarine sampling was conducted by us and by the Louisiana Department of Wildlife and Fisheries. *G. sanguineum* was observed in low numbers (10^2–10^3 cells/liter) at 62% of 16 sites sampled over the next month. Among the sampling areas were Calcasieu Lake, Calcasieu Pass, and a station just offshore of Calcasieu Pass on July 19, 1994. Although this was one of the areas implicated earlier as the source of the *G. sanguineum* bloom (Buzan[2]) along the west Louisiana shelf, *G. sanguineum* was present at the stations in the lake and the pass only at very low concentrations (10^2 cells/liter). Thus, around the time of the bloom and fish kill along the Louisiana-Texas border, the same species was widely distributed, sometimes, but not always, at high concentrations.

[2] Buzan, D. 1994. Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, TX 78744. Personal commun.

Distribution of *G. sanguineum* Along the Louisiana–Texas Coast 1989–93

G. sanguineum was observed in 7.5% of 1,962 samples taken in Louisiana and Texas coastal waters between 1989 and 1993. The rounded morphological variant that dominated in the June 1994 bloom was observed in some of these samples, although it was usually not as dominant as it was in the bloom. No cysts of the type seen after the incubations were noted in these routinely collected samples, although Steidinger et al. (1998) saw similar structures in water samples from the bloom.

G. sanguineum occurred more commonly in nearshore areas, especially those directly influenced by the plumes of the Mississippi or Atchafalaya rivers (Fig. 1). Thus, it is more likely to be observed along the Louisiana coast than along the Texas coast. It can occur at any time of the year (Fig. 3), but is present most frequently in the months of June through September. While its apparent absence in some months is due to a lack of sampling (Jan. and Nov.), it is sparse in other months for which adequate samples were collected. The sampling may have been concentrated in areas where *G. sanguineum* occurs less frequently.

Salinity measurements taken in the area of "tea-colored" water on the west Louisiana shelf in June 1994 ranged from 15 to 20‰ (Denton et al., 1998). Phytoplankton samples taken from the Louisiana and Texas coastal zones and Louisiana estuaries from 1989–93 show that *G. sanguineum* can tolerate salinities from 0.5 to 36‰ (Fig. 4), but is significantly more abundant at low salinities.

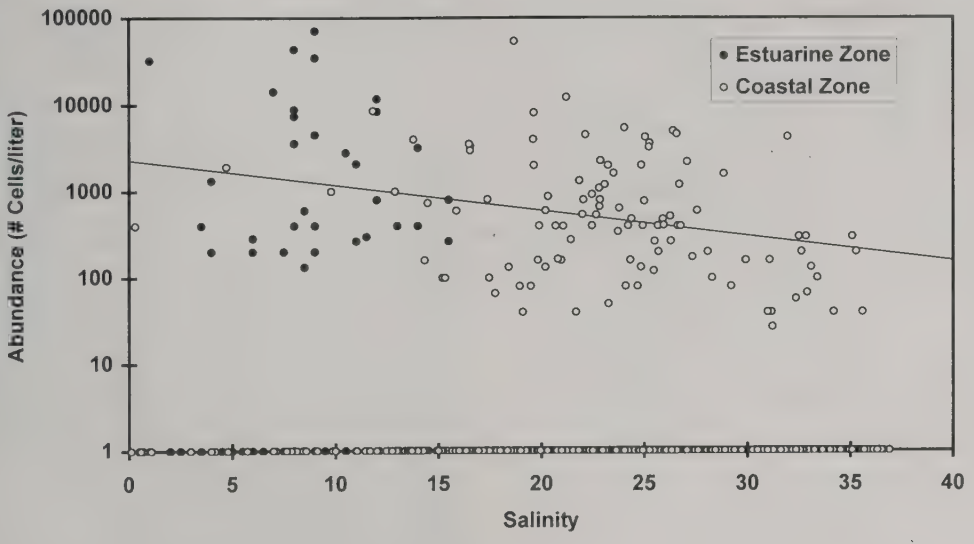

Figure 4

Numbers of *G. sanguineum* vs. salinity from 1989–93 on the Louisiana-Texas shelf and in 1993–94 from a Louisiana estuary. Samples with no *G. sanguineum* were assigned a value of one so they would appear on a semi-log plot. [For all the non-zero data, the Log Cell Number = –0.0291249 salinity + 3.3622; $r^2 = 0.1109$, n = 148; P-value of slope = 0.0004.]

Discussion

Association of *G. sanguineum* with Fish Kills

Two fish kills have occurred in the same location on the upper Texas/western Louisiana coasts, both associated with *G. sanguineum* (Harper and Guillen, 1989; this study). Several hypotheses have been proposed to explain this association. The first suggests that the fish kill was caused by low oxygen conditions, induced either by death, sinking, and decomposition of the bloom or diel vertical migration of the bloom organism and its respiration in bottom waters at night. The second hypothesis is that *G. sanguineum* produces ichthyotoxins which directly cause fish mortality. There is insufficient data obtained during this fish kill to determine if either of these mechanisms is the cause of the fish kill. Evidence from the literature supports the possibility that either or both could occur.

Mass sinking of dinoflagellates has caused hypoxic/anoxic events and mass mortalities elsewhere including one off the coast of Peru associated with a bloom of *G. sanguineum* (Dugdale et al., 1977). The *G. sanguineum*-associated fish kill in 1984 off the Texas-Louisiana coast was attributed to oxygen depletion due to decomposition of the algal bloom, although the evidence for low oxygen was indirect (Harper and Guillen, 1989). *G. sanguineum* is also well known for its strong vertical migration under some conditions (Kiefer and Lasker, 1975; Blasco, 1979; Cullen and Horrigan, 1981), although the possibility that its respiration in bottom waters at night could deplete the oxygen sufficiently to cause a fish kill has never been investigated.

G. sanguineum is not generally thought of as a toxic dinoflagellate, and in fact has been considered a good food source for a variety of marine animals (e.g., Lasker et al., 1970; Paffenhofer, 1970, 1971; Scura and Jerde, 1977). On the other hand, there are studies indicating it is a very poor food source, has negative effects for other marine animals, or is toxic (Cardwell et al., 1979; Tindall et al., 1984; Sellner and Olson, 1985; Bricelj et al., 1992; Turner et al., 1996), but no mechanism has been determined. Samples of *G. sanguineum* from the latest bloom and fish kill were not cytotoxic (Van Dolah[3]), but oyster samples tested with the mouse bioassay showed some toxicity (Denton et al., 1998). Given the widespread occurrence of this organism in coastal areas, its use as a food source in aquaculture, and the recurring bloom/fish kill association, further studies of its possible toxicity are necessary.

[3] Van Dolah, F. 1994. Marine Biotoxins Program, Charleston Laboratory, National Marine Fisheries Service, P.O. Box 12607, Charleston, SC 29422-2607. Unpubl. data.

Cysts and the Life Cycle of *G. sanguineum*

In the sample held for a month in the incubator in the light, structures similar to typical dinoflagellate cysts formed. None of the other dinoflagellate species present at the time the sample was put in the incubator would have formed a cyst of that size or structure. It is, of course, possible that some other species bloomed and formed cysts during the month when the sample was not examined. However, cyst formation by this species had been hypothesized previously. Voltolina (1993) demonstrated that sediment samples, taken from a lagoon on the west coast of Canada with recurring blooms of *G. sanguineum*, led to growth of vegetative cells. Despite a failure to isolate cysts, it was concluded that the blooms recurred because of reseeding from cyst beds. Moreover, Steidinger et al. (1998) described a similar cyst-like body in samples collected at the edge of the discolored water from the June 1994 bloom along the Louisiana-Texas coast. Thus, it seems likely that the observed structure was a cyst, although it's unclear what type of cyst. The red accumulation body is typical of a dinoflagellate hypnozygote. On the other hand, its rapid disappearance from the incubated sample, even in the dark at lower temperatures, argues that it is a temporary cyst.

The vegetative cells were surprisingly resistant to chemical degradation, much more so than the cyst-like structures. Since vegetative dinoflagellate cells are not usually subjected to standard palynological treatments, there is no way to know if this resistance is unusual. Further, it is difficult to extrapolate from resistance to chemicals in the lab to resistance to decomposition in the natural environment.

The *G. sanguineum* observed in this bloom has a number of characteristics which suggest that life cycle studies would be useful for understanding its distribution. The vegetative cell can occur in distinctly different morphological forms and is chemically quite resistant. It forms at least one type of cyst, which does not appear to be very persistent. Cysts or vegetative cells in sediments could provide the seed population for recurring blooms, but a better understanding of the life cycle is necessary before this hypothesis can be evaluated.

Predicting Blooms of *G. sanguineum*

G. sanguineum is often associated with water discoloration events and may be considered a Harmful Algal Bloom (HAB) species due to its association with mortality events. Blooms of many HAB species are thought to be increasing in coastal areas, perhaps as a result of increasing coastal eutrophication. Consequently, there is considerable interest in understanding the environmental conditions which lead to blooms, in order to predict their occurrence (WHOI, 1995).

In the northern Gulf of Mexico, *G. sanguineum* occurs primarily at low salinities near shore during the summer. Association with the Mississippi and Atchafalaya River plumes, which have much higher nutrient loads than other freshwater sources entering the Gulf (Turner and Rabalais, 1991; Rabalais, 1992) suggests that it may not specifically be the low salinity conditions, but perhaps the higher nutrient availability, that is stimulating *G. sanguineum* growth. This is supported by evidence that *G. sanguineum* blooms in high salinity upwelling areas, such as the coasts of Peru and California (Blasco, 1975). It also blooms aperiodically in the highly eutrophic Chesapeake Bay during the summer (Bockstahler and Coats, 1993). It appears to prefer periods of higher water stability and/or reduced wind stress, even in upwelling areas (Keifer and Lasker, 1975; Rojas de Mendiola, 1979; Robinson and Brown, 1983; this study). Further studies are needed to determine if its growth is stimulated by high nutrient availability. If that is the cause, blooms of this species are likely to increase simultaneously with coastal eutrophication.

Conclusions

G. sanguineum is a HAB species which is sometimes associated with fish kills, may produce cysts, and may be stimulated to grow when nutrient availability is high. Further study of its life cycle, possible toxicity, and nutrient requirements are necessary in order to predict blooms and their possible consequences.

Acknowledgments

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Fish Kills in the Northwestern Gulf of Mexico, 26 April–27 June 1994

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ABSTRACT

Two fish kills during May and June 1994 occurred in nearshore waters of the northwestern Gulf of Mexico from Freeport, Texas, east to Cameron, Louisiana. The number of fish killed exceeded 1,400,000. The causes of the fish mortality were never conclusively determined. Investigations during the incidents suggested that causes of the mortalities may have included a toxic algal bloom, low dissolved oxygen, and commercial fishing.

Biologists for the Texas Parks and Wildlife Department accessed aerial surveys, field observations, water chemistry analysis, laboratory toxicity bioassays, biological samples, histopathological and parasitological analysis, and biotoxin tissue testing to determine the cause of the fish die-offs. Aerial surveys located patches of discolored water where freshwater enters the nearshore Gulf of Mexico. Numerous dead fish were floating in and near the discolored water. Elevated dissolved oxygen and chlorophyll concentrations in areas of discolored water were consistent with phytoplankton bloom conditions. Biotoxin analysis of oysters collected from the Sabine Pass jetties indicated the presence of an unidentified toxin in oyster tissues. Histopathological and parasitological analyses were negative. Toxic substances were not detected in water.

Introduction

Texas Parks and Wildlife Department (TPWD) biologists investigate as many as 200 fish kills each year. Historically, low dissolved oxygen has caused the most fish kills, statewide. The largest kills in recent years have occurred in Texas coastal waters, resulting from rapid onset of extreme low temperatures and from toxic dinoflagellate blooms. Rapid drops in water temperatures to near freezing occurred three times, from 1983 to 1990, and resulted in die-offs of over 28 million fish.[1] A massive bloom of *Gymnodinium brevis* from Galveston, Texas, to the Rio Grande killed over 22 million fish in bays and the Gulf of Mexico, from August 1986 through January 1987 (Trebatoski, 1988).

During 1976, a fish kill involving more than 10 million fish was reported from the same area (TNRCC[2]). The 1976 kill was reportedly caused by a red tide. A fish kill in Gulf waters between Galveston and Freeport, Texas during the spring and summer of 1984 was attributed to hypoxia and possible elevated hydrogen sulfide which may have been caused by a massive bloom of *Gymnodinium splendens* (Harper and Guillen, 1989).

Seventeen fish kills have occurred along the upper Texas Gulf coast since 1970 (TNRCC[2]). Fourteen of these were between Galveston Beach, Galveston County, and Sea Rim State Park beach in Jefferson County. Sixteen of the 17 documented kills occurred during summer. Seven kills were attributed to mortality of the

[1] Mambretti, J. TPWD, 4200 Smith School Road, Austin, TX 78744. Unpubl. data.

[2] TNRCC. 1994. Fish kill report database retrieval. Texas Natural Resource Conservation Commission, P.O. Box 13087, Austin, TX 78711–3087.

bycatch from commercial fishing for shrimp and menhaden. Other causes of fish mortality included red tide (2 incidents) and spawning stress (1 incident). Causes for seven of the fish kills were not identified.

Federal, state, and local environmental investigators rarely have resources needed to adequately investigate Gulf fish kills. Consequently very limited data are available from the majority of these kills. Many of the kills are suspected to have resulted from commercial fishing for shrimp and menhaden which takes place during much of the spring and summer along the north Texas Gulf coast. Commercial fishing certainly has the capability to result in the accidental deaths of large numbers of fish. On 5 September 1994, 1.4 million menhaden and over 300 adult red drum washed ashore dead when the contents of a menhaden purse seine were lost just off the Gulf beach east of Galveston.

The first of the two fish kills reported in this document began in late April and early May 1994 and the second began during late June 1994. The first event included an estimated 650,000 fish, mostly hardhead catfish, *Arius felis*, and gafftopsail catfish, *Bagre marinus*. Sea turtle and bottlenose dolphin mortalities were elevated above normal during the same time and in the same locations as the dead fish.

The second event, which began during the last half of June 1994, killed over 800,000 of mostly bottom-feeding sciaenids. Estimates of dead fish during these incidents did not include fish that washed ashore on Louisiana beaches. Dolphin and sea turtle mortalities were not as high in the June fish kill as during the May fish kill. Visual observations and personal communications with State of Louisiana regulatory personnel suggested that unusually large numbers of dead fish were observed along western Louisiana's beaches during both the May and June incidents.

TPWD biologists investigated both kills to identify the cause(s) of mortality. Despite those efforts, insufficient data were collected for TPWD biologists to identify conclusively the cause(s) of the kills. Evidence suggests the most likely cause(s) was either toxicity from a toxic algal bloom, low dissolved oxygen, commercial fishing, or a combination of these.

Materials and Methods

May 1994

On 9 May 1994 Texas Natural Resource Conservation Commission (TNRCC) and TPWD personnel measured water quality and collected Gulf water samples approximately 1.6 km east of the Sea Rim State Park beach for chemical and phytoplankton analysis. One set of samples was collected and measurements were made in a patch of discolored water about 75 m long and 300 m wide. Other patches of discolored water were observed nearby. Dead fish were observed floating in the discolored water. A second set of samples was collected and measurements were made nearby but outside the area of visibly discolored water. Water depth was 2.4 m at both sites. Vertical profiles of water physicochemistry were made *in situ* with a multiparameter meter. Water samples were collected at depths of 0.3 m below the surface and approximately 0.3 m above the bottom. Water samples for chemical analysis were collected, preserved, and analyzed according to standard procedures (TNRCC[2]). Analyses for heavy metals and volatile organics were conducted at the Texas Department of Health (TDH) Environmental Chemistry Lab in Austin. Analyses for nutrients, chlorophyll, salts, and total and volatile suspended solids were conducted at the TNRCC laboratory in Houston. Beached dead fish were not extensively counted during the first incident.

June 1994

Dead fish were counted by TPWD biologists on 23, 26, and 27 June 1994 on the Gulf beach. Counts were made according to the American Fisheries Society Special Publication Number 24, "Investigation and Valuation of Fish Kills" (American Fisheries Society, 1992). Dead organisms were counted in six linear transects, totaling 320 m, along the beach from High Island east to Sea Rim State Park, a distance of 48 km.

Water quality testing for salinity and dissolved oxygen was conducted by TPWD biologists at several sites in the Gulf of Mexico in the area between Galveston and Sabine Pass. The measurements were taken both at the surface and near the bottom at each site.

Three oyster, *Crassostrea* sp., tissue samples were collected by TPWD biologists on 28 June 1994 from inside Sabine Pass at the Sabine Pilot station. Oyster tissues were analyzed by mouse bioassay following American Public Health Association procedures for biotoxin at the TDH laboratory in Austin.[3]

Results

May 1994 Fish Kill

On 2 May 1994, TPWD received citizen reports of dead hardhead catfish along the Gulf beach on Galveston Island. Similar reports and observations continued through the first week in May. On 7 May 1994, extremely large numbers of dead hardhead catfish washed

[3] Ordner, M. 1994. Texas Department of Health, 1100 W. 49th Street, Austin, TX 78756. Personal commun.

ashore at TPWD's Sea Rim State Park in Jefferson County. TPWD and U.S. Coast Guard staff conducted an aerial survey on 8 May 1994 which revealed approximately 36 kilometers of Texas beach lined with dead fish and several sea turtles. The aerial survey revealed an area of reddish discolored water offshore of Sabine Pass between Louisiana and Texas. Similar areas of discolored water were observed offshore of Rollover Pass in Texas and Cameron Pass in Louisiana. These areas of discolored water were also observed during aerial surveys conducted by TPWD personnel on 9, 11, and 12 May 1994. A TPWD biologist counted 574 hardhead catfish, 16 Gulf menhaden, *Brevoortia patronus,* and 7 Atlantic croaker, *Micropogonias undulatus,* on an area of beach at Sea Rim State Park, 7.6 m by 7.6 m, on 8 May 1994. Another TPWD biologist observed over 200 dead hardhead catfish on the Gulf beach near the mouth of the San Bernard River on the same date. TPWD staff seined in the surf at Sea Rim State Park during the morning of 9 May 1994, and collected relatively high numbers of apparently healthy hardhead catfish. One seine haul captured 125 fish.

In excess of 650,000 fish were estimated to have washed ashore on Texas' Gulf beach from Galveston Island east to Sabine Pass during the first two weeks in May 1994. The total area affected included the Gulf beach from the mouth of the San Bernard River in Brazoria County, Texas, east to Sabine Pass, Jefferson County, Texas, a distance of nearly 190 km. Estimates of dead fish included only the fish reported from the Gulf beach from Galveston east to Sabine Pass. The greatest concentrations of dead fish, equaling 75 dead fish/linear meter of beach, were found from Sea Rim State Park east to Sabine Pass. The majority of dead fish on Sea Rim State Park beaches washed ashore on 7 May 1994.

Hardhead catfish comprised the majority of the dead fish. Hardheads ranged in size from 15 to 41 cm and some were identified as gravid females. Dead Atlantic croakers were juveniles ranging from 10 to 15 cm. Gulf menhaden that were killed were primarily adults.

Water Quality

Dissolved oxygen in the discolored water ranged from 12.5 to 12.8 mg/l, top to bottom. In nearby waters that were not discolored, dissolved oxygen was 8.0–8.4 mg/l. The discolored water also had a pH of 8.4 compared to an average 8.0 in nearby water of normal appearance. Salinity was 16‰ at both sampling locations. Chlorophyll concentration from a depth of 0.3 m in the discolored water was 61 μg/l.[4] Analyses for volatile organic compounds and heavy metals in water samples from near the surface and bottom did not reveal elevated concentrations.

TPWD personnel conducted routine fish monitoring in Gulf waters near Sabine Pass on 18 April (8 sites), 4 May (3 sites), and 5 May (5 sites) 1994. Salinities at trawl sites during trawling ranged from 10–21‰. Dissolved oxygen concentrations near the bottom at trawl sites were 6.4–8.4 mg/l. Live, healthy fish were captured in all 16 trawls.

Bacteria, Virus, and Parasite Analysis

Three specimens each of moribund and healthy hardhead catfish, collected from Sea Rim State Park Gulf beaches by TPWD personnel on 8 and 9 May 1994, were analyzed at the Texas Agricultural Extension Service Fish Disease Laboratory for bacterial, viral, and parasitic infections.[5] All direct stains of internal tissues for bacteria were negative and no apparent lesions of microbial nature were observed. Parasitic lesions were absent and no important parasites were seen. Gills were reported reacting in a manner consistent with bacterial challenge. Internal tissues did not show evidence of bacterial challenge.

Laboratory Toxicity

Some of the water samples collected on 9 May 1994 (at 1640 hrs) by TNRCC and TPWD personnel from an area of discolored water off Sea Rim State Park beaches were analyzed for toxicity by the U.S. Environmental Protection Agency laboratory in Houston. They were analyzed using the nine-day chronic laboratory bioassay for sheepshead minnow, *Cyprinodon variegatus,* eggs and larvae.[6] The samples were collected 0.3 m below the surface and 0.3 m from the bottom. When compared to the control, the samples showed no significant effect.

June 1994 Fish Kill

An aerial survey conducted on 10 June 1994 by TPWD personnel revealed discolored waters in the Gulf near Sabine Pass and Cameron Pass. Each area was estimated to exceed two square kilometers. On 21 June 1994, the park manager reported large numbers of dead fish on the beach west of Sea Rim State Park.

[4] Graham, W. 1994. Texas Natural Resource Conservation Commission, 3870 Eastex Freeway, Suite 110, Beaumont, TX 77703. Personal commun.

[5] Johnson, K. 1994. Texas Agricultural Extension Service Fish Disease Laboratory, Texas A&M University, College Station, TX 77843. Personal commun.

[6] Hollister, T. 1994. U.S. Environmental Protection Agency, 10625 Fallstone Road, Houston, TX 77099. Personal commun.

TPWD conducted an aerial survey on 22 June 1994 and reported the areas of discolored water again near the mouths of Sabine and Cameron passes. Thousands of floating dead fish were observed during the 22 June aerial survey in nearshore Gulf waters between Sea Rim State Park and High Island. TPWD personnel trawled the bottom for 10 minutes in an area of discolored water near Sabine Pass on 18 June 1994 but did not capture any apparently stressed or dead fish.

A total of 5,485 dead fish and crabs, representing 16 species, were counted in transects along the beach from High Island east to Sea Rim State Park. Counts of dead animals were expanded to an estimate of over 820,000 dead fish and crabs on the beach.

The majority, over 96%, of fish were Atlantic croaker, silver perch, *Bairdiella chrysoura*, and star drum, *Stellifer lanceolatus*, ranging in size from 8 to 13 cm total length. Two percent of the dead fish were Atlantic spadefish, *Chaetodipterus faber*, with total lengths also measuring 8–13 cm. Atlantic bumper, *Chloroscombrus chrysurus*, bighead searobin, *Prionotus tribulus*, striped mullet, *Mugil cephalus*, inland silverside, *Menidia berylina*, Gulf menhaden, hardhead catfish, shrimp eel, *Ophichthus* sp., blue crab, *Callinectes* spp., purse crab, *Persephona* sp., spider crab, *Libinia* sp., spotted seatrout, *Cynoscion nebulosus*, and sand trout, *Cynoscion arenarius*, were also found dead on the beach.

Water Quality

Limited water quality tests were conducted by TPWD biologists in one area of discolored water near Sabine Pass on 22 June 1994 (Mambretti, personal commun.). Measurements were made at 1200 hrs in water 4.0 m deep. Salinity was 12‰ at the surface; 15‰ at the bottom. Dissolved oxygen was 15.4 mg/l at the surface; 9.2 mg/l near the bottom.

TPWD personnel conducted routine fish monitoring in Gulf waters near Sabine Pass on 14 June (8 sites), 18 June (5 sites), and 20 June (3 sites) 1994. Salinities at trawl sites were 7–21‰. Dissolved oxygen concentrations near bottom ranged from 2.6 to 8.8 mg/l. Live healthy fish were captured in all 16 trawls.

TPWD personnel also conducted routine water chemistry testing on 25 and 28 June 1994 in the Gulf of Mexico in the area between Galveston and Sabine Pass. At 17 sites, salinity ranged from 30 to 42‰ and dissolved oxygen concentrations in bottom waters was 0.2–9.3 mg/l. Dissolved oxygen levels were 1.0 mg/l or less at 8 of the sites.

Toxicity

Oyster tissues collected from Sabine Pass near the Sabine Pilot Station did indicate the presence of a toxin; however, the results were near the sensitivity limitations for the test procedure. The results for the three samples ranged from less than 8.7 Mouse Units to less than 13 Mouse Units.

Discussion

Two major fish die-offs occurred during 1994 along Gulf of Mexico beaches from Freeport to Sabine Pass, Texas. The first incident occurred during the first two weeks of May and the second during the third week of June. The estimated total number of dead fish on the beaches during both incidents was over 1,400,000. During May, over 90% of the dead fish were hardhead catfish. During the June incident, over 96% of the dead fish were the sciaenids, Atlantic croaker, star drum, and silver perch. Algal blooms, giving the water a reddish brown color, were observed near the passes from bays into the Gulf during both incidents.

TPWD field personnel made a large number of field observations and participated in several sampling efforts during May and June associated with the two fish kills. Negative results from the Fish Disease Laboratory for analysis of samples for bacteria, virus, and parasite infections suggest the May fish kill was not caused by a widespread bacterial, viral, or parasitic disease. Laboratory bioassays and chemical analysis of Gulf waters during the May incident failed to show either acute or chronic toxicity or the presence of heavy metals or man-made compounds in potentially toxic concentrations. Limited dissolved oxygen measurements prior to and during the May and June kills failed to reveal low levels of dissolved oxygen that would be lethal to fish. There was, however, no sampling of dissolved oxygen concentrations in the vicinities of the dead fish during early morning hours, when oxygen concentrations would be expected to occur at minimal levels.

Commercial fishing for menhaden and shrimp was observed during the May kill while commercial fishing for menhaden was observed during the June incident. Purse seining for menhaden and trawling for shrimp are known to result in the capture and subsequent mortality of nontarget species. Anecdotal information from beach users and TPWD biologists during the May incident suggested shrimping activity may have been conducted closer to the beach than in previous years. Observations of intensive shrimp trawling, combined with the awareness that mortality of nontarget species can result from trawling, suggest some of the dead fish observed during May 1994 resulted from commercial fishing.

It is unlikely commercial fishing contributed to observed mortality during the June 1994 incident. The relatively small size of the fish observed would have

allowed them to be harvested with any menhaden if they had been caught in a purse seine. The commercial shrimping season was closed during June 1994, making it unlikely that shrimping was responsible for fish mortalities during that incident.

Oyster tissues containing toxin were collected from the Sabine Pass area which experienced an algal bloom observed several times from the air during May and June. These data suggest a toxic algal bloom may have been present in Sabine Pass for some period preceding 28 June 1994.

Acknowledgments

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An Account of the 1994 Phytoplankton Blooms and Mass Mortalities of Marine Animals along the Western Louisiana and Northern Texas Coast, with Comparison to Similar Events of 1984

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ABSTRACT

Early summer phytoplankton blooms preceded mass mortalities of primarily demersal fishes and crustaceans from Calcasieu Pass, Louisiana, to the vicinity of Galveston, Texas, in 1984 and again in 1994. The 1984 event occurred in June and was evenly distributed along the affected coastline. The bloom was dominated by the dinoflagellate *Gymnodinium sanguineum* Hirasaka, not previously known to cause mortality. There ensued a late June mass mortality of demersal fishes and crustaceans, principally Atlantic threadfin, *Polydactylus octonemus*. The 1994 events featured separate phytoplankton blooms that developed adjacent to the estuarine passes. The dominant species of phytoplankton differed among the blooms and did not include toxic species previously known to cause mass mortality. Two temporally separate mass mortalities occurred: An early May mortality, principally affecting hardhead catfish, *Arius felis*, and a late June mortality principally affecting Atlantic croaker, *Micropogonias undulatus*. Due to the lack of highly toxic, mass mortality-producing phytoplankters, it seems unlikely that dinoflagellate toxins caused the mortalities in either year. The demersal nature of nearly all fishes and crustaceans involved in both years suggests that low dissolved oxygen levels in bottom waters might be a possible cause. Comparison is made between the 1984 and 1994 events and a phenomenon known as a "jubilee." Additional possible causes or contributing factors, such as commercial by-catch, disease, pollution, and underwater explosions, are also considered.

Review of the Mass Mortality Event of 1984

Phytoplankton Bloom

Harper and Guillen (1989) reported the occurrence of a brown-colored phytoplankton bloom that appeared along the western Louisiana and upper Texas coast in June 1984. The bloom was initially observed from the Galveston beachfront on 10 June. An aerial survey on 19 June revealed patches of discolored water extending southwestward from Calcasieu Pass to the southwestern end of Galveston Island. These extended seaward to a

distance of 10 km (Fig. 1) and persisted for two weeks until disappearing on 27 June.

During the early stages of the bloom, Harper and Guillen (1989) reported coastal salinities as low as 9 ppt. They indicated that this condition resulted from high spring discharge from the mouths of the Mississippi and Atchafalaya Rivers, which lie to the northeast, associated with westerly coastal currents and southeasterly (onshore) wind patterns. They detected hypoxic (<2 ppm) water masses at depths below 5 m off Galveston. In addition, they observed a layer of black, hydrogen sulfide-laden, anaerobic silt covering the sediment surface just prior to the disappearance of the bloom. This layer extended seaward to a distance of 200 m. Samples of the discolored water were provided to L. A. Loeblich who identified the dominant phytoplankter as the dinoflagellate *Gymnodinium splendens* Lebour, presently known as *Gymnodinium sanguineum* Hirasaka.

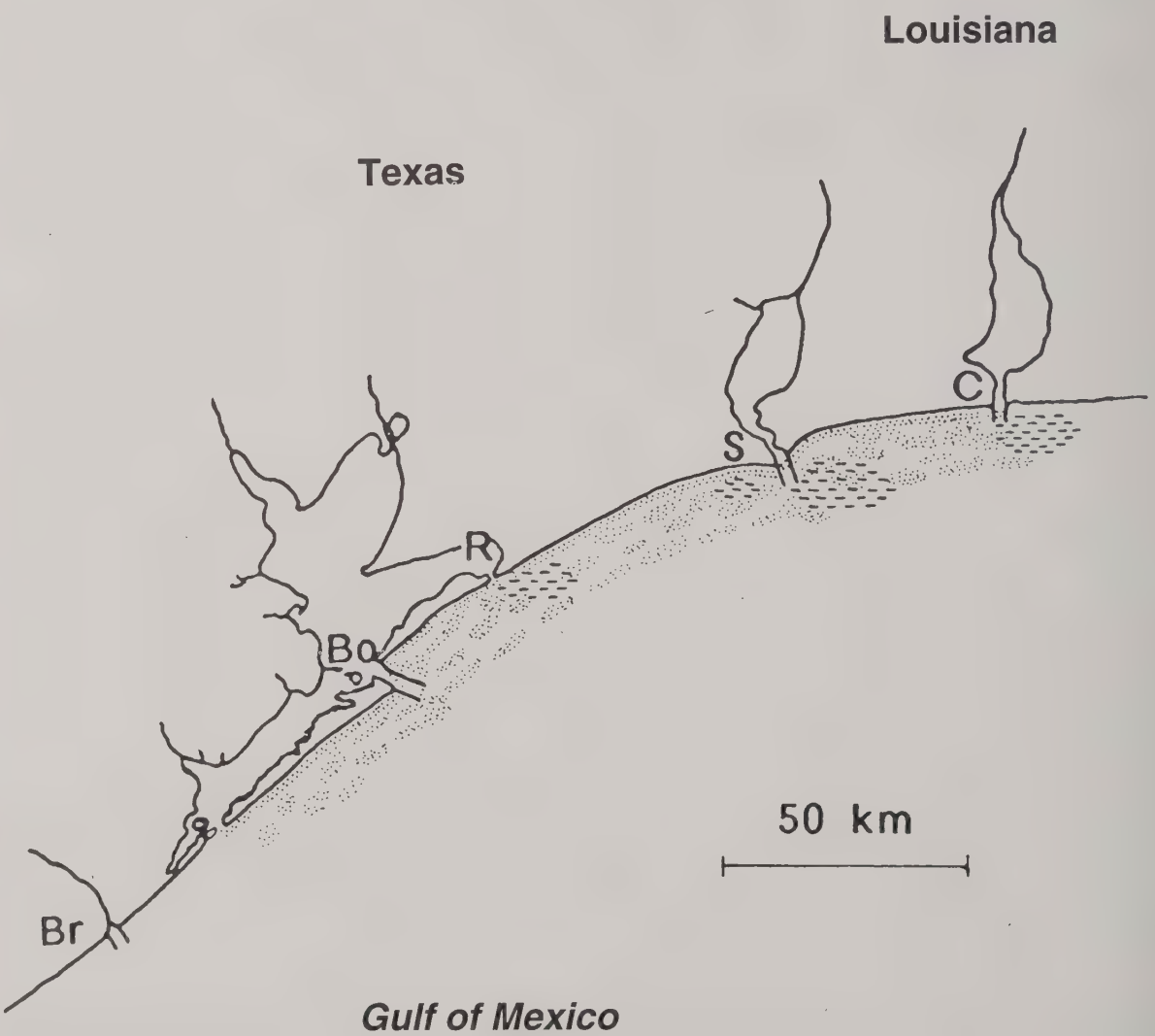

Figure 1

Distribution of phytoplankton blooms along the western Louisiana and northern Texas coast in June 1984 (stippled area) and as initially observed in May 1994 (dashed areas). Br = Brazos River, Bo = Bolivar Roads, R = Rollover Pass, S = Sabine Pass, C = Calcasieu Pass.

Mass Mortality

On 23 June, Harper and Guillen (1989) recorded a mass mortality of predominantly demersal fishes and crustaceans 13 days after the bloom was first observed. Dead organisms occurred along the entire bloom-affected shoreline (Fig. 2) and continued to accumulate on shore for five days. The mortality ceased with the disappearance of the discolored water. The Atlantic threadfin, *Polydactylus octonemus*, accounted for 80% of the estimated thirteen million individuals killed. The remainder consisted of several other species of primarily demersal fishes and crabs.

An Account of the 1994 Phytoplankton Bloom and Mass Mortality

Bloom Development and Composition

The 1994 bloom was observed on 1 May and consisted of successive bands (0.4 hectare) of reddish-colored water parallel to the shoreline. Plumes consisting of many bands extended in an easterly direction from the mouths of Calcasieu, Sabine, and Rollover passes (Fig. 1). Within a few days, the plumes expanded to a distance of 3.2 km from shore and began to extend southward from the mouths of the passes, then eventually southwestward. Blooms persisted in the Calcasieu and Sabine Pass areas until late June but the bloom in the Rollover Pass area disappeared by the end of May.

Uncharacteristically low coastal salinity readings of less than 20 ppt were recorded. Daytime dissolved oxygen (DO) readings varied considerably but low readings (2.8 and 4.1 ppm) were recorded off the Sabine Pass jetties on 1 July.

Samples of discolored water were obtained from the separate blooms at Calcasieu, Sabine, and Rollover passes. Light and electron microscopic examination (Steidinger et al., 1998) revealed that blooms at Sabine and Rollover passes were dominated by the dinoflagellate *Gymnodinium sanguineum* Hirasaka. Also present were the dinoflagellate *Prorocentrum minimum*, the raphidophyte *Heterosigma* sp., euglenophytes, and silicoflagellates. The bloom at Calcasieu Pass, however, was quite different in its composition, being dominated by *Heterosigma* sp., with lesser concentrations of other dinoflagellates, diatoms, cryptomonads, and prymnesiophytes.

Gymnodinium sanguineum, a well-known cosmopolitan species, is not known to be toxic. Although some species of *Prorocentrum* are known to cause paralytic shellfish poisoning, *P. minimum* has no history of toxicity. *P. minimum* has been recorded in bloom proportions in large areas of Mississippi Sound and adjacent waters (Perry and McClelland, 1981) with no attendant mortality of marine organisms. *Heterosigma akashiwo* (Hada) is reported to produce red tides in the western Pacific which are toxic to juvenile salmon (Yamochi, 1983). There are no previous records of mortality caused by any species of *Heterosigma* in the Gulf of Mexico. Furthermore, *Heterosigma* sp. was detected in significant numbers only in the Calcasieu Pass area bloom in 1994 and was not recorded in 1984.

Mass Mortality of Demersal Fishes and Crustaceans

Two separate mass mortalities of predominantly demersal fishes and crustaceans occurred in conjunction with the 1994 blooms. The events were temporally separate and spatially different (Fig. 2).

The first mortality became evident on 2 May, one day after discolored waters were initially observed at the mouths of the passes. Dead fishes and crustaceans were found floating in coastal waters and accumulating on the shore from Calcasieu Pass to Bolivar Roads (Fig. 2). The event resulted in deaths of an estimated 630,000 catfishes, predominantly hardhead catfish, *Arius felis*, with a small but not-precisely-determined number of gafftopsail catfish, *Bagre marinus*, as well. In addition, approximately 19,000 Atlantic croaker, *Micropogonias undulatus*, and lesser numbers of other demersal fishes and crustaceans perished (Table 1). An estimated 650,000 organisms succumbed over two weeks, ending on 15 May.

The second mass mortality occurred over an 8-day period, 22–30 June 1994. Demersal fishes and crustaceans accumulated on the shoreline from Sabine Pass eastward, a distance of 45 km (Fig. 2). This event resulted in the deaths of an estimated 800,000 individuals but, unlike the first mortality, Atlantic croaker was the dominant species affected. Other species occurring in the second event were similar in type to those observed in the first, except that catfishes were not observed.

Comparison of the 1984 and 1994 Events

Abiotic Conditions

Currents in the 1984 bloom area moved westward along the coast, bringing low salinity water from Louisiana (Harper and Guillen, 1989). In 1994, the sources of low-salinity water appear to have been local passes (Calcasieu, Sabine, and Rollover). This is supported by the following: (1) Plumes of discolored water (Fig. 1) appeared at the mouths of the passes and were initially observed extending seaward in an easterly direction and (2) the phytoplankton blooms in 1994 differed in species composition from pass to pass, suggesting that each of the three blooms was nurtured individually by local waters from an adjacent pass. In 1984, by contrast,

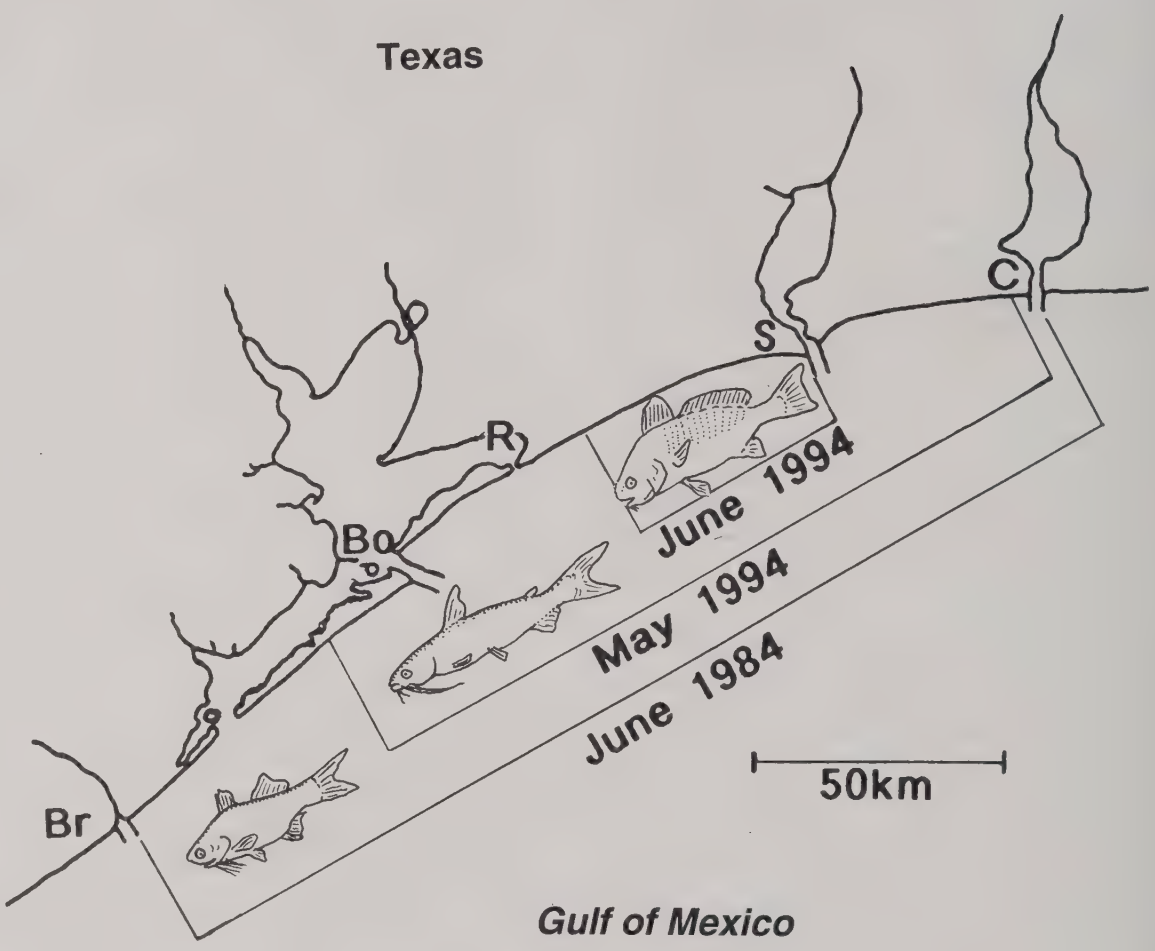

Figure 2

Distribution of mass mortalities of fishes and crabs along the western Louisiana and northern Texas coast in 1984 and 1994. The dominant organisms for each mortality are shown from left to right: *Polydactylus octonemus, Arius felis, Micropogonias undulatus.* Br = Brazos River, Bo = Bolivar Roads, R = Rollover Pass, S = Sabine Pass, C = Calcasieu Pass.

blooms were apparently nurtured by a single westward-flowing water mass.

Low salinity coastal water (less than 20 ppt), probably resulting from recent freshwater runoff, was recorded during the 1984 and 1994 events. Normally, salinities along the northern Texas coast exceed 20 ppt during the months of May, June, and July (Pullen et al., 1971; Harper and Wardle, 1972-present, personal observ.).

Southeasterly (onshore) wind patterns prevailed during May and June 1994 (personal observ., present investigators). These may have served to hold low salinity water masses close to shore, as in 1984 (Harper and Guillen, 1989).

Low dissolved oxygen (DO) readings (<2 ppm) were recorded in association with the bloom in 1984. In 1994, somewhat low DO readings of 2.8 and 4.1 ppm were recorded. Although these are not hypoxic levels, they are surprisingly low considering that they were taken during daytime in bloom areas of presumably intense photosynthetic activity and oxygen production.

Table 1
Composition and estimated magnitude of mass mortalities of fishes and crustaceans on the western Louisiana and northern Texas coast in 1994.

26 April–15 May 1994	Percent	22–30 June 1994	Percent
Arius felis	85	*Micropogonias undulatus*	90
Bagre marinus	5	*Stellifer lanceolatus*	4
Micropogonias undulatus	3	*Bairdiella chrysura*	2
Brevoortia patronus	<1	*Chaetodipterus faber*	2
Sciaenops ocellata	<1	*Larimus fasciatus*	<1
Pogonias cromis	<1	*Prionotus tribulus*	<1
Ophichthus gomesii	<1	*Cynoscion arenarius*	<1
Callinectes spp.	<1	*Arius felis*	<1
Total = 656,220	100	*Mugil cephalus*	<1
		Echiophis sp.	<1
		Callinectes spp.	<1
		Libinia sp.	<1
		Persephona sp.	<1
		Total = 827,130	100

Hydrogen sulfide accumulation on bottom sediments was detected in 1984. No comparable sediment sampling was performed in 1994.

Bloom Development and Composition

The phytoplankton blooms of 1984 and 1991 were similar in regard to their seasonal timing and general location. They differed, however, as to uniformity and pattern (Fig. 1). The bands of discolored water in the 1984 bloom were distributed uniformly along the shoreline from Calcasieu Pass to the southwestern end of Galveston. The 1994 event, however, featured several isolated blooms that remained in the vicinity of the estuarine passes throughout their duration. In 1984, the single bloom lasted only 17 days. The 1994 blooms persisted for about 60 days in the vicinity of Calcasieu and Sabine passes.

The 1984 bloom was dominated by a single species, the dinoflagellate *Gymnodinium sanguineum*. *G. sanguineum* was also a key species in the 1994 event in blooms at Sabine and Rollover passes. At Calcasieu Pass, however, the dominant species was the raphidophyte *Heterosigma* sp.

Mass Mortality of Demersal Fishes and Crustaceans

The mass mortalities of 1984 and 1994 were similar in that both involved primarily demersal fishes and crustaceans. In 1984 only a single mass mortality event was recorded, while in 1994 there were two temporally separate and spatially different mass mortalities. The chief victims of the 1984 mortality were Atlantic threadfin while the primary victims of the first and second 1994 mortalities were hardhead catfish and Atlantic croaker, respectively.

An estimated 13 million fishes and crustaceans were killed in 1984 whereas total deaths in 1994 were only one-tenth that number. The Atlantic threadfin that perished in the 1984 mortality were far smaller in body size than the hardhead catfish and Atlantic croaker killed in the 1994 mortalities, thus the total biomass that perished in 1984 may be comparable to that killed in 1994.

Discussion

Harper and Guillen (1989) concluded that hypoxia or hydrogen sulfide poisoning, rather than dinoflagellate toxins, were the most likely causes of the 1984 mortalities. They noted that the dominant phytoplankter had not been previously associated with mass mortalities, and that the list of species killed included primarily demersal types. A broader spectrum of species would be expected to be killed by a less selective red tide toxin such as the "brevetoxin" produced by the dinoflagellate *Gymnodinium breve* (Davis). This toxin has a history of causing periodic mass-mortalities of fishes along the Texas coast (Wilson, 1956; Texas A&M Univ. Sea Grant, 1987).

The 1984 and 1994 events are similar in having occurred in the same geographic area at more or less the same time of the year. They are also comparable in having been associated with low salinity and, possibly, low DO levels. The occurrence of phytoplankton blooms followed by mass mortality of predominantly demersal

fishes and crustaceans is also similar. The two events differ, however, as to the apparent sources of the low salinity waters in which blooms developed. They differ also in patterns of bloom distribution, taxonomic composition of the bloom organisms, and bloom duration. The numbers and types of demersal nekton affected during the two events were also quite different.

The saxitoxin-producing dinoflagellate *Alexandrium monilatum* (Howell) (formerly *Gonyaulax monilata* Howell) is known to have caused blooms along the Galveston coast in previous years. Those were followed by mass mortalities of demersal fishes and macroinvertebrates (Wardle et al., 1975). Although the 1984 and 1994 bloom waters were not tested for the presence of saxitoxin, it is unlikely that saxitoxin was a factor because *Alexandrium monilatum* cells were not recorded in plankton samples taken during either event. Other dinoflagellates were found (as summarized in Steidinger et al., 1998), however, none, including *Gymnodinium sanguineum* which was prominent in both the 1984 and 1994 events, is known to have sufficient toxin-producing capability to have caused such mortality.

The 1984 and 1994 events along the Louisiana-Texas coast share some characteristics with a previously-reported northern Gulf of Mexico inshore phenomenon known as a "jubilee." Loesch (1960) and Gunter and Lyles (1979) observed that jubilees involve concentrations of live demersal fishes and crustaceans in shallow estuarine waters. These concentrations are due to the presence of hypoxic conditions in adjacent deeper waters where these animals normally reside. The hypoxia results either from high biological oxygen demand generated by decomposing organic matter, or from nighttime respiration of phytoplankton in bloom areas. Jubilees may be associated with blooms of non-toxic dinoflagellates and characteristically occur in waters of less than 20 ppt salinity. Hypoxic conditions associated with jubilees are often alleviated before mass mortalities occur. Other jubilees are more prolonged, causing mass mortalities of fishes and crustaceans; some jubilees occasionally cause deaths of pelagic forms, i.e. menhaden, *Brevoortia patronus*.

The events of 1984 and 1994 along the western Louisiana and northern Texas coast cannot be regarded as jubilees in the classic sense for they did not occur in inshore waters and were of much larger scale in area, duration, and magnitude of mortality. The 1984 and 1994 events do, however, share some characteristics with jubilees, i.e. the dominant phytoplankton bloom species were non-toxic. The species affected in 1984 and 1994 are comparable to those affected by jubilees. In addition, low salinity water (<20 ppt) was associated with the 1984 and 1994 events, also characteristic of jubilees. Finally, relatively low dissolved oxygen levels were observed during both events. The few sporadic readings taken in 1994, however, did not adequately demonstrate widespread hypoxia.

Other causes for the 1984 and 1994 mass mortalities must, therefore, be considered. For instance, were mortalities caused by larger scale seasonal hypoxic events in offshore bottom water? Renaud (1986) and Rabalais et al. (1994) have reported such events in this area. Another possible cause or contributing factor is fishing industry by-catch. Mortality of nontarget demersal nekton species by commercial fishermen commonly occurs each year in the affected area (Caillouet et al., 1991). The resulting patterns of deposition of organisms on the shore, however, are usually much more intermittent and of far less magnitude than those observed in the 1984 and 1994 mortalities.

Toxicity due to industrial pollutants normally would be expected to affect both pelagic and demersal species. The possibility of the presence of bottom-distributed toxic substances of industrial origin in one or both mortality years cannot, however, be discounted.

Mortality due to disease normally would be expected to be confined to a single species or to a few closely related species. One of the authors (Denton) observed a mass mortality of catfishes, *Arius felis* and *Bagre marina*, along the northern Texas coast in August 1995. The mortality occurred over a period in late summer during which no phytoplankton blooms were observed. This suggests the possibility that disease might have played a significant role, not only in the 1995 mortality, but in the first (May) mass mortality of catfishes in 1994.

Anthropogenic underwater explosions associated with the offshore activities of oil and gas industries are also common in this area. Such activity can result in fish mortality and could be a possible contributing factor.

The primary cause(s) of the 1984 and 1994 mass mortalities along the western Louisiana and northern Texas coasts thus remains essentially unknown at this time. It is hoped that this documentation and comparison of these events in the present report will be useful in comparisons with similar events occurring in this area in the future.

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Assessment of the Involvement of Algal Toxins in the 1994 Texas Fish Kills

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ABSTRACT

Water samples collected from discolored patches east of Galveston, Texas, during May–July 1994 were examined for the presence of toxic algae and algal toxins. The dominant algal species varied between water samples, with the presence of *Gymnodinium sanguineum* being the only common denominator. Methanol extracts were analyzed for toxicity by mouse bioassay and by *in vitro* cytotoxicity, and further characterized for diarrhetic shellfish toxins (DSP) and brevetoxin-like activity using pharmacologic assays. None of the algal samples were toxic by mouse bioassay or cytotoxicity assay. Oysters collected from Sabine Pass during an intense bloom of *Gymnodinium sanguineum* and analyzed for *in vitro* cytotoxicity were also negative. No DSP toxins were found. However, all algal and oyster samples displayed some activity in a receptor binding assay for brevetoxin-like compounds, which was not observed in negative controls. Results of toxicity assays are discussed relative to the algal species present.

Introduction

During April and May 1994 large numbers of demersal gafftopsail, *Bagre marinus,* and hardhead, *Arius felis,* catfishes washed ashore along the upper Texas coast. The onset of catfish mortalities generally coincided with an increase in the numbers of dolphin and sea turtle strandings along the Texas coast. The occurrences of fish kills similarly coincided with the presence of extensive and patchy areas of "coffee-colored" water located from the shoreline to approximately ten miles offshore. Blooms of dinoflagellates cause discoloration of water, resulting in red to brown patches of the nature observed here. Furthermore, a number of dinoflagellate species have been implicated in fish kills (Anderson, 1994) and marine mammal mortalities (Geraci et al., 1989). Depending on the algal species present, fish kills may result from anoxic conditions, physical damage to gills, or production of specific neurotoxins. This report summarizes the results of assays for algal toxins which were performed on water samples collected from within and outside of discolored patches during May and June, and on oysters collected in the presence of an intense algal bloom in Sabine Pass during June.

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Methods

Water Sample Collection

Water samples (8 L) were collected via Coast Guard helicopter from coffee-colored patches approximately 1 mile offshore from Sabine Pass and Rollover Pass on 12 May 1994. Subsamples were fixed in Lugol's iodine for species identification. The remainder of the sample was stored at room temperature for 48 hours before analysis in the laboratory. Similarly colored water samples collected on 3 June offshore from Sabine Pass and on 5 July from the Galveston ship channel by Texas Parks and Wildlife (Winston Denton) were shipped to Charleston Laboratory for analysis.

Water Fractionation

Water samples were filtered through 250 μm and 20 μm Nitex[1] plankton screens. The fraction retained by the 20 μm filter (containing most phytoplankton) was sonicated in 10 ml methanol for 2 minutes, followed by centrifugation to remove particulate debris. The methanol was then evaporated to dryness and the sample dissolved in 100μl fresh methanol for analysis.

Oyster Samples

Oyster extracts were obtained from the Texas Public Health Department (Mike Ordner). These samples were extracted using the American Public Health Association (APHA) standard method (Greenberg and Hunt, 1984) and provided in cottonseed oil. An additional sample of oysters collected from Sabine Pass on 5 July was extracted in our laboratory using the APHA protocol. In the presence of 5 g NaCl and 1 ml concentrated HCl, 100 g shellfish homogenate was boiled, followed by extraction with 400 ml ethyl ether. The ether phase was evaporated to dryness, and the residue brought up in 9 ml methanol for analysis. Control oysters collected in Charleston, S.C., were analyzed in parallel.

Mouse Bioassay

Methanol extract (50 μl) was diluted to 5 ml with 0.9% saline containing 1% Tween 80. Two-fold serial dilutions were then made and 0.5 ml injections were administered intraperitoneally into female ICR strain mice (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) weighing approximately 20 g. Four dilutions were tested, with four mice injected at each dilution. Results are expressed as (+) or (–) based on mouse symptomology (incoordination, hind limb paralysis), indicative of neurointoxication.

Cytotoxicity

General cytotoxicity was determined by the method of Van Dolah and Ramsdell (1996). GH_4C_1 rat pituitary cells were plated in 0.1 ml Hams F10+ medium in 96-well plates at a concentration of 0.5×10^6 cells/ml. Sample extracts were then serially diluted, added to triplicate wells, and incubated 18 hours at 37°C. For determination of viability, 15 μl of the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/ml in PBS), was added and the cells were incubated for 4 hours at 37°C. The cells were then solubilized by addition of 10% sodium dodecyl sulfate (SDS) in 0.1 N HCl and absorbance at 570 nm determined using a 96-well plate reader.

Brevetoxin (PbTx) Receptor Binding Assay

[^{3}H]PbTx binding competition assays were carried out in 96-well polystyrene plates by the method of Van Dolah et al. (1994). For generation of PbTx-3 competition curves, 140 μl rat brain synaptosome preparation (1 mg/ml protein) were incubated in the presence of 35 μl [^{3}H] PbTx-3 (5 nM; Chiral Corp., Miami, Fla.) and increasing concentrations of unlabeled PbTx-3 (10^{-6} to 10^{-11} M) in the presence of 50 mM HEPES buffer (pH 7.4), 130 mM choline chloride, 5.5 mM glucose, 0.8 mM $MgSO_4$, 5.4 mM KCl, 1 mg/ml bovine serum albumin (BSA), and 0.01% Emulphor-EL 620. For analysis of algal extracts, 5 μl extract was diluted with 30 μl sample buffer and added in place of the PbTx-3 standard. The mixture was then incubated at 4°C for 1 hour, followed by filtration onto a 96-place filter mat and addition of solid scintillant. The 96-place filter mat was counted directly in a microplate scintillation counter.

Phosphatase Inhibition Assay

The presence of okadaic acid-like compounds was assessed by the ability of algal extracts to inhibit protein phosphatase activity *in vitro* (Ingebritsen et al., 1983), where the activity of protein phosphatase 2a was measured by its ability to release ^{32}P from a ^{32}P-labeled substrate phosphorylase a. Protein phosphatase 2a (Upstate Biotechnology, Saranac, N.Y.) was incubated in the absence of or presence of algal extracts and ^{32}P-

[1] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1
Toxicity results on water samples collected.

Location	Date	Mouse bioassay	Cytotoxicity
Cameron	12 May	neg	neg
Rollover	12 May	neg	neg
Sabine (1) Dark water	3 June	n.d.[1]	neg
Sabine (2) Clear water	3 June	n.d.	neg
Sabine (3) Dark water	3 June	n.d.	neg
Galveston Ship Channel	5 July	neg	neg

[1] n.d. = not determined.

phosphorylase a at 30°C for 15 minutes. The reaction was halted by addition of ice cold trichloroacetic acid (TCA, 10%). Phosphorylase a was then precipitated by centrifugation and ^{32}P released was quantified by counting an aliquot of the supernatant by liquid scintillation spectroscopy. Inhibition of the amount of ^{32}P released relative to untreated controls indicates the presence of phosphatase inhibitor.

Results and Discussion

Water Samples

Phytoplankton identification and toxicity testing were carried out on water samples collected on three dates, at approximately one month intervals, during the occurrence of discolored water patches. Discolored water samples were first collected by helicopter on 12 May 1994 from Cameron, Louisiana, and Rollover Pass, Texas, approximately one mile offshore. Both water samples were of low salinity, the Cameron sample at 16‰ and Rollover Pass at 20‰. The water from Cameron contained a mixed estuarine heterotrophic assemblage of dinoflagellates (see Steidinger et al., 1998), rather than a monotypic bloom. The sample was dominated by *Heterosigma* c.f. *akashiwo*, which has been known to cause fish kills by unidentified mechanisms. In contrast, similarly colored water collected the same day approximately one mile offshore from Rollover Pass was dominated by *Gymnodinium sanguineum*. The phytoplankton fractions collected in the 20 μm size filter mesh were negative for toxicity by mouse bioassay and by *in vitro* cytotoxicity assays (Table 1).

Additional samples were provided by Texas Parks and Wildlife Dept. on 3 June 1994 offshore from Sabine Pass, from both within and outside of discolored water patches. The major microalgal species in two samples from within the discolored water included dinoflagellates *Gymnodinium sanguineum*, *Dinophysis caudata*, *Prorocentrum minimum*, and euglenoids *Eutreptiella* spp., while algae cells in the sample from outside the discoloration were sparse. The material collected in the 20 μm fraction from all three samples were negative for toxicity by mouse bioassay and by *in vitro* cytotoxicity assays (Table 1).

Due to growing concerns regarding the potential for human health impacts associated with the persistence of discolored water along the Texas coast, and the presence of *Dinophysis* sp. in these samples, the 3 June 1994 samples were analyzed for the presence of diarrhetic shellfish poisoning (DSP) toxins. Analysis of DSP toxins was based on the action of DSP toxins to inhibit protein phosphatase 2a. No phosphatase inhibitory activity was observed in the samples from discolored water. Certain *Dinophysis* sp. are producers of DSP toxins, and low levels of DSP toxins have been identified in samples of *Dinophysis* collected from Alabama waters (Dickey[2]). However, there is no evidence that DSP toxins may cause fish kills and no incidents of DSP have been recorded in the Gulf of Mexico to date.

A third sampling of discolored water was carried out by Texas Parks and Wildlife on 5 July 1994 from Galveston shipping channel. This sample, dominated by *G. sanguineum*, also tested negative for toxicity in mouse and *in vitro* cytotoxicity assays (Table 1).

Gymnodinium sanguineum Hirasaka is an estuarine dinoflagellate which has been known to cause extensive red tides, and fish kills in certain cases. Nevertheless, this species is an important food source for some zooplankton, as well as larval (Lasker et al., 1970) and adult (Rojas de Mendiola, 1979) anchovy, *Engraulis mordax*.

Gymnodinium sanguineum is considered to be synonymous with *Gymnodinium nelsoni* Martin and *Gymnodinium splendens* Lebour. It is worth noting that even though its morphology is highly variable, some researchers still question whether *G. sanguineum* is synonymous with *G. splendens* based on the original description of the latter species. In conditions similar to those experienced in 1994, a June 1984 bloom of *G. splendens* at Galveston was linked to extensive kills of two demersal fish species, threadfin, *Polydactylus octonemus*, and croaker, *Micropogonias undulatus* (Harper and Guillen, 1989). Similarities between the incidents are time of year, high volume runoff conditions, and wind direction. Since *G. sanguineum* is a strong vertical migrator, it does particularly well in stratified water columns. The primary mechanism by which *G. sanguineum* is believed to cause kills is through hypoxia and hydrogen sulfide formation during the decay of a bloom (Robinson and Brown, 1983; Rojas de Mendiola, 1979). The presence of these conditions was noted during the 1984 fish kill.

[2] Dickey, R. 1994. Fisheries Research Branch, FDA, P.O. Box 158, #1 Iberville, Dauphin Island, AL 36528. Personal commun.

This species has also been reported as being responsible for oyster mortalities through clogging of gills (Nightingale, 1936). It has not been reported to produce a toxin, however a *G. sanguineum* extract at high concentration has been observed previously to cause low level toxicity in mice (Dickey[2]). Because of concern for potential human health impacts associated with the current *G. sanguineum* bloom, we analyzed the 20 μm fractions from all discolored water samples for the ability to inhibit brevetoxin binding in a receptor binding competition assay. We found that all sample extracts caused a partial inhibition of brevetoxin binding. However, further characterization of the receptor interaction is necessary before any conclusions can be made. *Gymnodinium sanguineum* is not believed to produce brevetoxin-like compounds. Our laboratory is currently assessing the toxicity of *G. sanguineum* in culture.

Oyster Samples

Extracts of oysters collected from reddish colored water in Sabine Pass on 28 June 1994, concurrent with a fish kill, were provided to us by Texas Dept. of Health, along with a control extract of oysters from Lavaca Bay, Texas, located west of the region impacted by the discolored water. All extracts were mildly toxic when tested by Texas Dept. of Health using the APHA mouse bioassay protocol (Ordner[3]), exhibiting borderline mouse toxicity, indicated as a "+" in Table 2. The extracts were tested in our laboratory for *in vitro* cytotoxicity (Table 2). All samples were negative in the cytotoxicity assay. Additional oysters were collected in Sabine Pass during an intense *G. sanguineum* bloom on 5 July 1994 by Texas Parks and Wildlife and shipped to Charleston Laboratory for analysis. These were extracted by a modification of the APHA protocol, in which they were solubilized in methanol rather than cottonseed oil. These samples were also negative in the cytotoxicity assay. Control oysters collected from Charleston Harbor, Charleston, S.C., were analyzed in parallel and showed no toxicity in either assay. Because of the results observed in the algal samples, we also analyzed the oyster samples for the ability to inhibit brevetoxin binding to the voltage dependent sodium channel, using a receptor binding competition assay (Van Dolah et al., 1994). We found that all oyster samples from Texas, including the "control" oysters from Lavaca Bay, caused a partial inhibition of brevetoxin binding in the assay, whereas control oysters from Charleston Harbor, S.C., had no inhibitory activity in the receptor assay. However, as in the case of the algal extracts, the nature of the brevetoxin binding inhibition observed in the oyster extracts from Texas must be further characterized before any conclusion regarding toxin production can be made.

[3] Ordner, Mike. 1994. Seafood Safety Division, Texas Dept. of Health, 1100 W. 49th St., Austin, TX 78756-3199. Personal commun.

Table 2
Toxicity results on oyster samples.

Location	Date	Mouse[1] bioassay	Cytotoxicity
Lavaca Bay(#24)	28 June	+	neg
Sabine Pass(#25)	28 June	+	neg
Sabine Pass(#26)	28 June	+	neg
Sabine Pass(#27)	28 June	+	neg
Sabine Pass	5 July	n.d.[2]	neg

[1] Data from Texas Health Dept.
[2] n.d. = not determined.

Summary

One or more algal blooms appear to have occurred on the upper Texas coast during May–July 1994, based on the spatial and temporal patchiness of the discolored water. However, given the diversity of dominant algal species identified in water samples obtained from similar-looking areas of discoloration, the involvement of a unialgal bloom in the fish kills is not definitive. *Gymnodinium sanguineum* appears to be the only common denominator among samples tested, although it was not consistently present in bloom proportions. Results of toxicity assays on all algal samples were negative by mouse bioassay and *in vitro* cytotoxicity assays. No DSP activity was detected in samples containing *Dinophysis* sp. Oysters collected from Sabine Pass during a bloom of *G. sanguineum* displayed borderline mouse lethality, but were negative in *in vitro* cytotoxicity assays. The potential for the production of sodium channel specific activity in *G. sangiuneum* is currently under investigation.

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Patterns of Bottlenose Dolphin, *Tursiops truncatus*, Strandings in Texas

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ABSTRACT

During the spring of 1994, large numbers of bottlenose dolphins, *Tursiops truncatus,* washed ashore along the upper Texas coast. The majority of these carcasses were in an advanced state of decomposition, indicating that death had occurred some time earlier, possibly offshore. Despite intensive efforts to determine the cause of death by traditional pathological examinations, no conclusions could be drawn. Eventually, through the use of polymerase chain reaction (PCR) techniques, the Armed Forces Institute of Pathology was able to determine that a large proportion of the animals had an active morbillivirus infection and that this was likely the cause of death. Compared to normal years, increased numbers of dead dolphins began washing up on beaches during December 1993, peaking in March and April 1994 when a total of 171 dolphins were retrieved and continuing through May 1994. Of this total, 89% were retrieved between Matagorda Bay and the Louisiana state line. The actual impact on the coastal bottlenose dolphin population may never be known because of a lack of robustness in the population estimates, however, ongoing surveys of the resident populations in Galveston, Matagorda, and Corpus Christi Bays do not indicate any decline in local abundance.

Introduction

Bottlenose dolphins, *Tursiops truncatus,* inhabit temperate and tropical waters in all of the world's oceans (e.g. Würsig and Würsig, 1979; Cockcroft and Ross, 1990; Corkeron, 1990; van Waerebeek et al., 1990) and are the most common coastal marine mammal species found in the Gulf of Mexico (Blaylock et al., 1995). This species exploits a wide variety of habitats (Kenny, 1990; Shane, 1990; Fertl, 1994; Blaylock et al., 1995) and has been described as having two distinct forms, or ecotypes; coastal and offshore (possibly two distinct species—see Curry, 1997). These two forms are recognized to differ in physiology, ecology, and morphology (Duffield et al., 1983; Hersh and Duffield, 1990; Mead and Potter, 1990; Curry, 1997). The coastal population of bottlenose dolphins in the southeastern United States appears to be composed of resident stocks that reside year round in local bays, as well as a coastal transient stock which migrates along the coast on a seasonal basis (e.g. Bräger et al., 1994; Blaylock et al., 1995; Mate and Worthy, 1995).

Because of its position at the top of the food chain, various researchers have described this species as one that could be useful in monitoring our coastal environment. To this end, it is one of the most widely studied species of marine mammals in the world. Many investigators have examined life histories, behavior, feeding habits, movement patterns, and energetics, as well as a wide variety of other parameters including contaminant accumulation, parasites, and diseases. These latter areas of investigation are facilitated by the activities of various stranding networks throughout the world (Odell, 1985; Wilkinson and Worthy, 1999).

The Texas Marine Mammal Stranding Network (TMMSN) was founded in 1980 with the goal of recovering all dead and live stranded marine mammals which come ashore along the approximately 1,000 miles of Texas coastline. It is a volunteer organization, comprised of both lay people and professional biologists, as well as employees of federal agencies (National Marine Fisheries Services and National Parks Service personnel), state agencies (Texas Parks and Wildlife Depart-

ment, Texas A&M University, and the University of Texas), and private organizations (Sea World of Texas and Texas State Aquarium). The TMMSN is divided into six regions, each of which has a Regional Coordinator. These Regional Coordinators relay data and samples to the State Operations Coordinator who is based in Galveston, Texas. This system allows for efficient and effective coverage of the entire Texas coast. During the history of the TMMSN, the bottlenose dolphin has accounted for approximately 96% of recovered animals with the remainder comprised of 19 additional species of marine mammals (Table 1), ranging from manatees, *Trichechus manatus* (Fernandez and Jones, 1990) to sperm whales, *Physeter macrocephalus* (Jones, 1988; Tarpley and Marwitz, 1993).

During the period December 1993 through May 1994, large numbers of dead bottlenose dolphins washed ashore on the Texas coast. This paper describes, and attempts to explain, the temporal and geographic distribution of strandings of that unusual mortality event. These types of analyses can lead to a better understanding of the causes of unusual mortality events (e.g. Bodkin and Jameson, 1991).

Materials and Methods

Reports of stranded dolphins were relayed to the TMMSN either directly from the public or through governmental agencies, and a recovery team was dispatched. A coding system was used to identify the degree of freshness of a recovered carcass; code 1 refers to a live stranding, code 2 is very fresh (less than 8–10 hours postmortem), and carcasses decline in freshness to a code 5 animal which is a mummified carcass or skeletal remains. Such a coding system identifies the limitations placed on investigators in terms of identifying the cause of death. Once an animal was recovered, a condition code was assigned, information on gender and basic morphometrics was obtained, if possible, and the exact location of the stranding was noted. This was not possible in the case of many of the code 4 or 5 animals due to advanced decomposition.

Recently dead, fresh animals (condition code 2 or early code 3) were immediately returned to our necropsy facility in Galveston where a complete necropsy was performed. Animals which were more decomposed, designated as late 3, 4, or 5 condition codes, were necropsied on the beach, where voucher specimens were collected as well as samples for toxicological and virological studies. Some of these latter studies were undertaken by the Armed Forces Institute of Pathology (e.g. Kraft et al., 1995; Lipscomb et al., 1996). All animals were examined for any indication of fisheries interactions or obvious injuries.

Table 1
Numbers of representatives of non-*Tursiops* species which have stranded along the Texas coast between 1980 and 1996.

Order Cetacea	
Balaenoptera acutorostrata	1
Stenella coeruleoalba	4
S. attenuata	5
S. longirostris	5
S. clymene	15
S. frontalis	7
Stenella sp.	17
Steno bredanensis	2
Lagenodelphis hosei	1
Globicephala macrorynchus	1
Peponocephala electra	2
Physeter macrocephalus	3
Pseudorca crassidens	3
Grampus griseus	3
Feresa attenuata	5
Mesoplodon europaeus	4
Ziphius cavirostris	3
Kogia breviceps	8
K. simus	8
Kogia sp.	8
Order Sirenia	
Trichechus manatus	4

Results and Discussion

During the early years of the Texas Marine Mammal Stranding Network, an expansion in numbers of participants and public awareness resulted in a greater degree of coastal coverage and increasing numbers of recovered dolphins (Fig. 1). From 1986 through the present, the intensity of coverage has remained constant and therefore annual differences should reflect actual differences in stranding rates and distribution (Wilkinson and Worthy, in press). During normal stranding years, the TMMSN recovers approximately 130.7 ± 17.3 (± 95% CI) dolphins (Fig. 1) with non-*Tursiops* animals accounting for 4.2 ± 1.2% of total strandings in an average year (Fig. 1). This mean is based on numbers of dolphins that stranded in 1986–89, 1991, 1993, and 1995. There have been four years in which abnormally high numbers of dolphins have been recovered from Texas beaches: 1990, 1992, 1994, and 1996 (Fig. 1). Increased numbers of animals in each of these unusual years were solely a result of large numbers of bottlenose dolphins coming ashore rather than increased reporting effort.

Typically, dolphin strandings follow a seasonal pattern in Texas with more than 60% of animals coming ashore during the period February through April (Fig. 2).

Numbers of recovered dolphins decline during the summer months and begin to increase again in December and January (Fig. 2). In an average year, the TMMSN recovers 9.4 ± 2.2 dolphins in January, increasing to 38.0 ± 12.0 dolphins in March and subsequently declining to 2.8 ± 1.9 dolphins in June (Fig. 2). While this general pattern held true for 1993–94, the absolute numbers were significantly higher. A significant increase (t-test, $P < 0.05$) above normal levels was first noted in December 1993 and a return to normal did not occur until June 1994. During the peak months of March ($n = 90$) and April ($n = 81$), up to ten dolphins were being recovered in a single day.

During March and April 1994, a total of 171 animals were retrieved from the entire Texas coast, but 151 (89%) of those were collected from beaches east of Matagorda Bay and west of the Louisiana state line (Fig. 3). Despite regular vigilance by the public and several agencies, including ground and aerial beach surveys, animals were frequently discovered washing ashore as code 4 animals. Normally, code 4 and 5 animals account for less than 35% of recovered animals.[1] In January 1994, 50% of the recovered animals were either condition code 4 or code 5. This proportion increased considerably in February (70%) and remained high through March (65%) and April (53%). These high proportions of code 4 and 5 animals made identification of sex and the measurement of any morphometrics difficult.

Discoveries of these badly decomposed dolphins the day after a beach survey was performed suggests that animals were dying offshore and floating for days before drifting onto the beach. Taking into consideration ambient water (55–65° F) and air temperatures (45–65° F) (TMMSN records), it would take approximately 7 to 10 days for an animal to decompose to condition code 4. This suggests the possibility that animals may have died offshore and washed onto the beach after some period of time. During the period from 1 March until mid-April there was significant down coast and onshore surface water movements,[2] and onshore winds, which resulted in several research drifter buoys

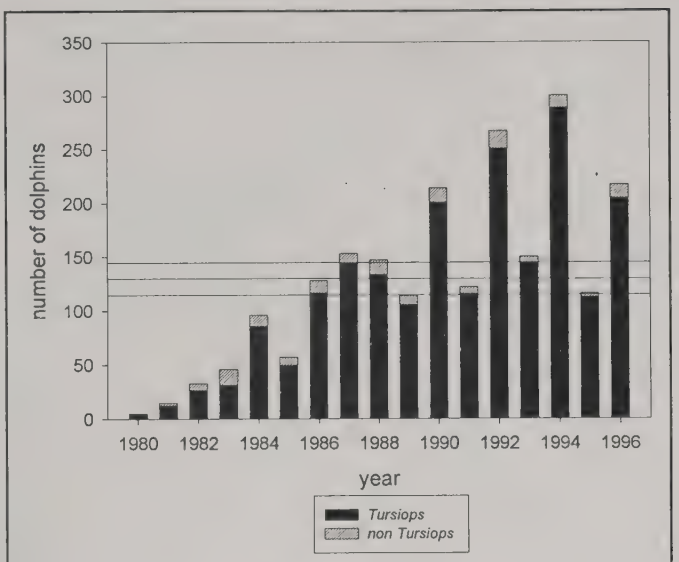


Figure 1

Total number of cetacean strandings recovered annually, 1980–96. *Tursiops truncatus* accounts for approximately 96% of all strandings. Horizontal lines refer to the average number of strandings (± 95% CI) recovered in a "normal" year (as defined by those years, 1986–89, 1991, 1993, 1995, in which an "unusual" event did not occur). Increasing numbers of animals recovered between 1980 and 1986 are a result of increased coverage of the coast by the TMMSN.

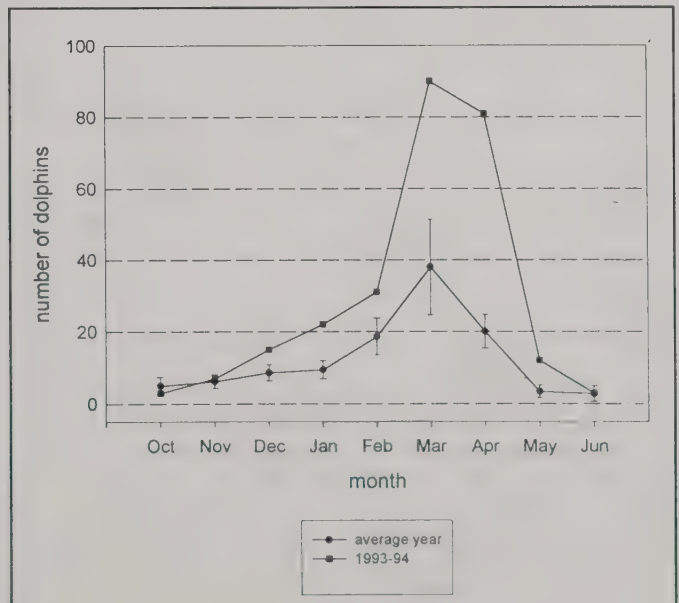


Figure 2

Mean (± SD) monthly strandings during "normal" years (as defined in Fig. 1) and the actual numbers of dolphins which stranded during 1993–94.

[1] Worthy, G. 1994. Texas Marine Mammal Stranding Network, 5001 Avenue U, Suite 105, Galveston TX 77551. Unpubl. data.

[2] Nowlin, W. 1994. Department of Oceanography, Texas A&M University, College Station, TX 77843. Personal commun.

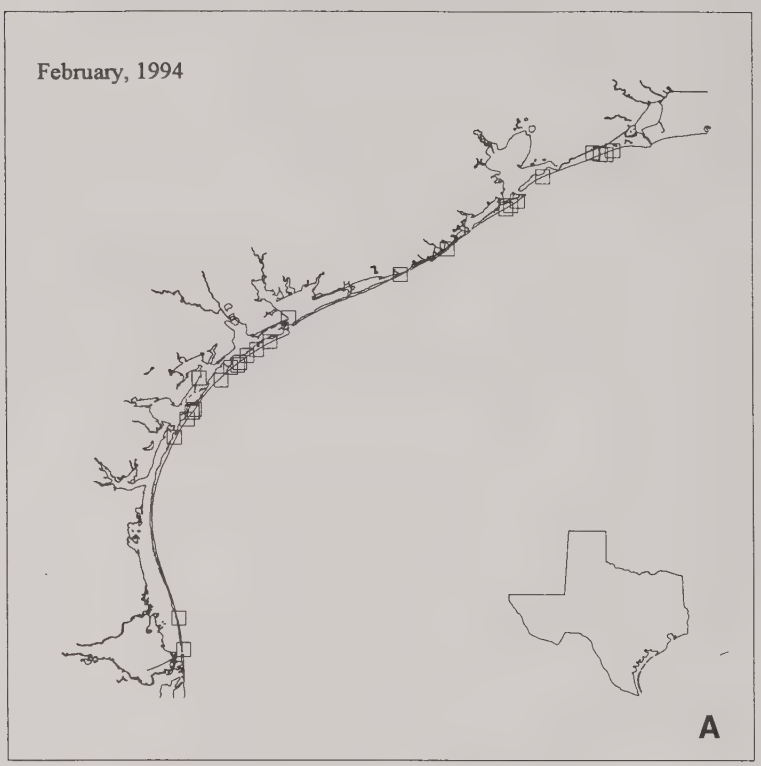
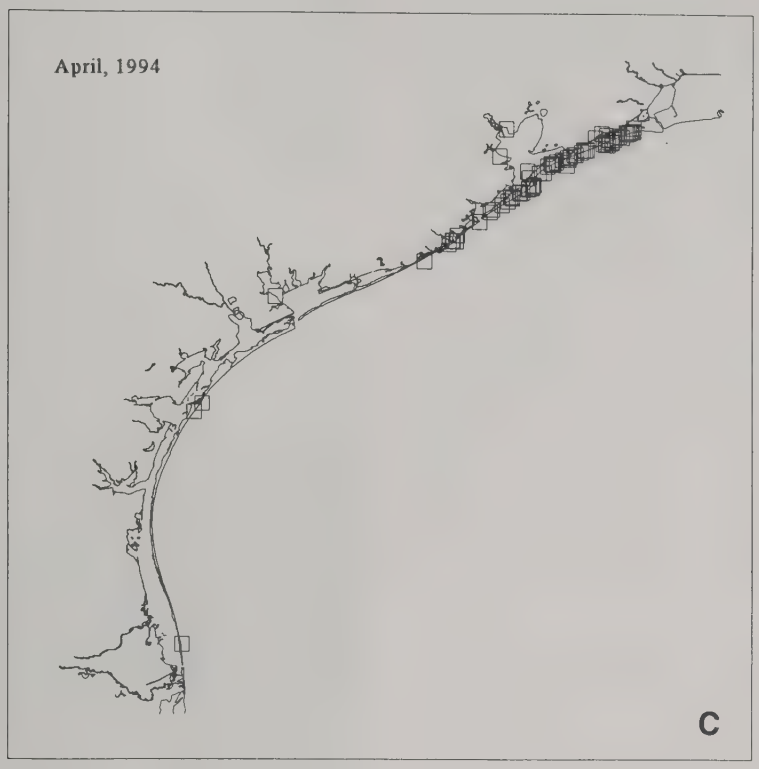


Figure 3
(Left and facing page) Geographic and temporal distribution of *Tursiops* strandings along the Texas coast: (A) February 1994, (B) March 1994, (C) April 1994, (D) May 1994. Each point is an individual dolphin.


April, 1994
C

May, 1994
D

washing ashore east of Galveston at the beginning of April and into May. The surface current patterns along the coast between Sabine Pass and Galveston Bay were atypical during this period and, in conjunction with the observed strong south and southeast winds, could result in the surface flow being driven into shallow water.[3] These data suggest that some animals may have died offshore of western Louisiana and eventually drifted ashore in east Texas. The time course of strandings does not suggest a discrete period for the mortality event, but rather a protracted period of several weeks (Fig. 3).

A total of three code 1 (live-stranding) animals and six code 2 dolphins were retrieved during the time course of this event. All three live animals tested negative for the presence of active morbillivirus and survived (Bull and Worthy, 1995), two of which were subsequently released and satellite-tracked (Mate and Worthy, 1995). The remaining animal was deemed too young to be released. None of the tested code 2 animals (two animals were not tested because they stranded near the Mexican border and were not likely part of the unusual event) showed any clinical evidence of morbillivirus[4] nor did they test positive using PCR techniques (Lipscomb et al., 1996).

An examination of length data collected from those animals which were intact suggest that 22.8% (46/202) of recovered dolphins were young-of-the-year (<110 cm), 10.4% (21/202) were approximately 2 years of age (>110–<130 cm), 27.7% (56/202) were between the ages of 2 and 5 years old (130–<230 cm), and the remaining 39.1% (79/202) had lengths in excess of 230 cm and are considered as sexually mature adults (Fig. 4) (Fernandez, 1992). Relative proportions of length categories found in the 1994 data are similar to those reported by Hansen[5] and Fernandez (1992) for bottlenose dolphins in Texas that stranded during the period 1983–90.

One problem in interpreting the sex ratio data relates to the difficulty in assessing the sex of a badly decomposed code 4 or 5 animal. It is often easy to identify males under these circumstances because, during the bloating process, the penis is frequently extruded. The sex of an animal that lacks an extruded penis cannot, however, be assumed to be female. This results in a large number of animals not being identified as to their sex (45%), and the sex ratio of those which are identified being skewed toward males (67% male:33% female). This should not be taken as meaning there were a greater number of males dying.

Two previous unusual mortality events have occurred in the Gulf of Mexico, the first during 1990 and the second in 1992. A total of 214 dolphins (201 *Tursiops*) were recovered from Texas beaches in 1990 and 267 dolphins (245 *Tursiops*) were recovered in 1992. In 1990, 23 dead dolphins were discovered on a single day in east Matagorda Bay after an unusually severe cold weather system moved through the area (Miller, 1992). No definitive cause of death was ever identified for animals which died elsewhere in the state or throughout the Gulf of Mexico during that year (Hansen, 1992), although a morbillivirus epizootic has recently been suggested based on preserved samples (Duignan et al., 1996). The remaining 178 dolphins collected during 1990 are still significantly greater than the number of animals retrieved during a normal year and were part of a Gulf-wide event.

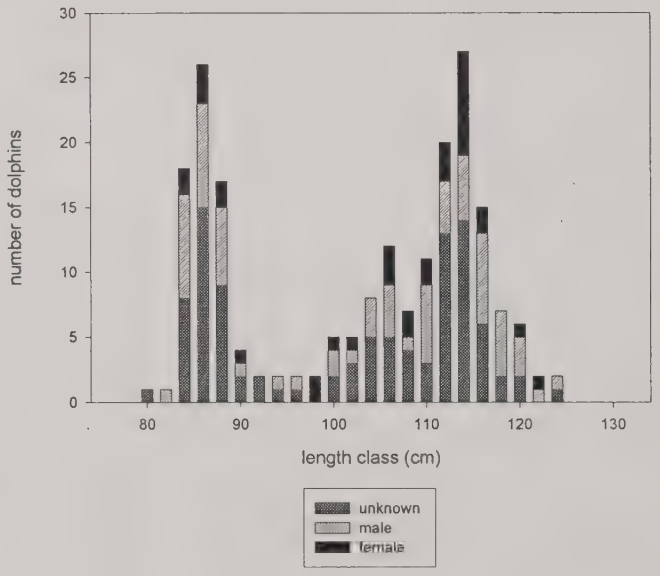


Figure 4
Numbers of dolphins collected during 1994 as a function of length category. Each bar is divided into males, females, and animals of unknown gender. Animals with lengths greater than 240 cm are considered to be adults (Fernandez, 1992).

[3] Zimmerman, R. 1994. A preliminary report on mortalities of marine animals in Texas during the spring of 1994. NMFS, SEFSC Galveston Laboratory, 4700 Avenue U, Galveston TX 77551.

[4] Cowan, D. 1994. Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555. Unpubl. data.

[5] Hansen, L. (ed.). 1992. Report on investigation of 1990 Gulf of Mexico bottlenose dolphin strandings. SEFSC, Miami Laboratory Contribution MIA-92/93-21, 219 p.

The 1992 mortality event was restricted to the Matagorda Bay area and occurred after a series of unusually heavy winter rains. Although no definitive cause was identified, one working hypothesis was that agricultural run-off contributed to it (Colbert et al., in press). A morbillivirus epizootic was not implicated because of low seroconversion rates observed in live animals (Duignan et al., 1996), which were captured as part of a National Marine Fisheries Service health assessment operation undertaken during the summer of 1992. Duignan et al. (1996) incorrectly reported that 220 dolphins died around Matagorda Bay during March and April 1992. The actual number for that time period was 119; the remainder of the 267 animals that were recovered in 1992 came from throughout the state over the balance of the year.[6]

Because of the inability to perform a thorough necropsy, there is considerable difficulty in assessing actual cause of death in animals which are as decomposed as the majority of the 1994 dolphins were. In code 4 animals, it is often impossible to even identify major organs. The utilization of polymerase chain reaction (PCR) techniques to identify the presence of active morbillivirus in lung tissue (Lipscomb et al., 1994b, 1996) was a major advance in the determination of cause of death. Some dolphins which washed ashore in Texas were so badly decomposed that nucleic acids could not even be amplified from the tissues which were collected (Lipscomb et al., 1996). In those animals for which amplification was possible, Lipscomb et al. (1996) discovered that in excess of 60% (29/57) of examined dolphins tested positive for the presence of active morbillivirus. If this proportion is extrapolated to the total number of dolphins recovered, these "infected" dolphins would account for all of the observed increase above normal stranding rates seen during early 1994.

Although increased numbers of strandings were not being reported in Florida when the initial morbillivirus infected animal stranded alive there in June 1993 (Lipscomb et al., 1994a), that single stranding event preceded an increased stranding rate throughout the Gulf of Mexico during the balance of 1993 and the first five months of 1994. Increased numbers of dolphins started coming ashore in Alabama from July through December 1993 and subsequently in Mississippi from August through December 1993 (Lipscomb et al., 1996). There was no organized stranding network operating in Louisiana and therefore we have no concept of what was happening there. Texas dolphins were found to be infected with morbillivirus throughout the upper Texas coast, regardless of the age of the dolphin (Lipscomb et al., 1996), and the temporal and geographic distribution of strandings during 1993–94 were consistent with a westward spread of the virus along the Gulf coast.

This recent outbreak of morbillivirus in the Gulf of Mexico is the same disease which resulted in the deaths of 18,000 harbor seals in northwestern Europe in 1988 (Kennedy, 1990; Markussen, 1992; Heide-Jorgensen et al., 1992), as well as thousands of striped dolphins in the Mediterranean in 1990 (Aguilar and Raga, 1993; Calzada et al., 1994). Recently a morbillivirus outbreak was implicated, using preserved tissues, as the cause of the 1987–88 U.S. Atlantic coast epidemic that resulted in a tenfold increase in the mortality of bottlenose dolphins (Lipscomb et al., 1994b) and the disease has been described in several other species found in the western Atlantic Ocean (Daoust et al., 1993; Duignan et al., 1995).

Since the proportion of animals which die at sea and which eventually wash ashore is unknown, it is virtually impossible to calculate the actual impact of this unusual mortality event. Minimum population estimates for the western Gulf of Mexico coastal stock is 3,499 dolphins (CV = 0.21), the offshore stock consists of 5,618 dolphins (CV = 0.26), and estimates of resident populations for Galveston, Matagorda, and Corpus Christi Bays total fewer than 500 animals (Blaylock et al., 1995). Ongoing surveys of resident populations of bottlenose dolphins found in these bays do not suggest any impact of the 1994 event on local populations[7] (Blaylock et al., 1995). Due to inadequate knowledge of the stock structure and/or population numbers (Blaylock et al., 1995), the true impact on the transient coastal and/or offshore populations may never be known.

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[6] Worthy, G. 1994. Texas Marine Mammal Stranding Network, 5001 Avenue U, Suite 105, Galveston TX 77551. Unpubl. data.

[7] Weller, D., and B. Würsig. 1994. Marine Mammal Research Program, Texas A&M University, 4700 Avenue U, Bldg. 303, Galveston TX 77551. Unpubl. data.

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Sea Turtle Strandings along the Texas Coast, 1980–94

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ABSTRACT

During 1980–94, 3,283 stranded sea turtles (2,929 dead, 354 alive) were found along the Texas coast, including 1,139 Kemp's ridley, *Lepidochelys kempii,* 1,524 loggerhead, *Caretta caretta,* 258 green, *Chelonia mydas,* 170 hawksbill, *Eretmochelys imbricata,* and 57 leatherback, *Dermochelys coriacea,* turtles and 135 unidentified turtles. More turtles stranded during 1994 than during any year from 1980 to 1993. There were various sources of mortality during 1980–94. A large percentage of the strandings were probably due to incidental capture in shrimp trawls. Fewer stranded turtles were found during the Texas Closure (when some Gulf of Mexico waters off Texas were closed to shrimp trawling) than before and after the closure. During 1994, temporal and spatial distributions of the strandings coincided with nearshore shrimping and the numbers of stranded turtles were inversely related to Turtle Excluder Device enforcement.

Stranding records reveal the importance of Texas waters for Kemp's ridley turtles. This species comprised an increasing percentage of the strandings along the Texas coast; 48% percent of the 527 documented strandings during 1994 were Kemp's ridley turtles. Fewer, but larger, Kemp's ridley turtles stranded along the middle and lower Texas coast. Based on analyses of digestive tract contents, some Kemp's ridley turtles in Texas are consuming the by-catch discarded by shrimp trawls. Turtles may concentrate in areas of shrimping to exploit that easily obtainable food and thereby become vulnerable to capture by trawls.

Introduction

Stranded sea turtles are dead or live turtles that wash ashore or are floating; live floating turtles are generally in a weakened condition (Schroeder, 1988; Teas, 1993). The Sea Turtle Stranding and Salvage Network (STSSN) was established in 1980 to document strandings of marine turtles on U.S. beaches along the Gulf of Mexico, Atlantic Ocean, and Caribbean Sea (Schroeder, 1988). Because not all stranded turtles are detected, the numbers reported by the STSSN are considered to be minimum estimates of the total number of strandings (Schroeder, 1988; Teas, 1993).

Stranded sea turtles along the Texas coast (Rabalais and Rabalais, 1980; Whistler, 1989) include all five species that occur in the northwestern Gulf of Mexico: Kemp's ridley, *Lepidochelys kempii,* loggerhead, *Caretta caretta,* green, *Chelonia mydas,* hawksbill, *Eretmochelys imbricata,* and leatherback, *Dermochelys coriacea,* turtles.

Several investigations have been conducted to determine causes of the strandings and to study various attributes of the stranded animals (Heinly et al., 1988; Plotkin and Amos, 1990; Shaver, 1990a; Shaver, 1990b; Caillouet et al., 1991; Shaver, 1991; Plotkin et al., 1993; Sis et al., 1993). Factors that have been implicated in sea turtle strandings along the Texas coast include collision with boat propellers (Whistler, 1989), oiling (Whistler, 1989), entanglement (Plotkin and Amos, 1990), ingestion of fishing gear and marine debris (Plotkin and Amos, 1990), and incidental capture during recreational or commercial fishing (Whistler, 1989; Caillouet et al., 1991). Although explosives to remove oil and gas platforms during 1986 (G'tschlag and Renaud, 1989) caused at least 51 strandings, an intensive observer program was

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instituted in 1987 to prevent such occurrences, and no strandings have been attributed to that source since then (Gitschlag and Renaud, 1989; Richardson, 1989; Gitschlag, 1992). Forty-seven turtles stranded as a result of hypothermic stunning in 1989 (Shaver, 1990a, 1990b). Twenty-five hatchlings and post-hatchlings stranded in 1986 and 53 in 1990 (Shaver, 1992), probably from being washed ashore by strong winds and currents.

Incidental capture in shrimp trawls has been identified as a significant source of sea turtle mortality along the Texas coast and in other areas in the southeastern United States (Magnuson et al., 1990). To reduce trawl-related mortality, Turtle Excluder Devices (TED's) have been phased into mandatory usage in Texas waters since 1990. Since 1981, some Gulf of Mexico waters off Texas have been closed to shrimp trawling for 6–8 weeks to allow shrimp to grow to a larger size prior to harvest (termed Texas Closure). This closure has encompassed the entire month of June and varying portions of May and July. Reductions in the number of stranded turtles located during the Texas Closure were noted prior to the adoption of Turtle Excluder Device regulations (Magnuson et al., 1990).

Sea turtle strandings along the Texas coast continued after the implementation of TED regulations and reached unprecedented levels during 1994. This study was done to examine trends in the numbers of strandings and affected animals, particularly in 1994 and the critically endangered Kemp's ridley turtle, and to determine possible causes of the strandings. To provide comprehensive protection of threatened and endangered turtles, anthropogenic causes of strandings must be identified and reduced (Magnuson et al., 1990).

Materials and Methods

Sea Turtle Stranding and Salvage Network

Procedures for locating and documenting stranded turtles in Texas were described by Whistler (1989). Stranded turtles were located by network participants in response to information from beach visitors and during systematic surveys conducted in some areas of the state. Few systematic surveys were conducted before 1986. Systematic surveys to locate stranded turtles were most comprehensive during 1986–90. From 1986 to 1989, the National Marine Fisheries Service supplemented voluntary efforts by STSSN participants with systematic, year-round surveys on the Texas coast (Caillouet et al., 1991). From 1990 to 1994, the survey areas and frequency were reduced. Little effort was made to survey in remote regions of the Texas coast such as the Matagorda Peninsula, San Jose Island, South Padre Island, and inshore areas. The survey area and frequency decreased along the upper Texas coast, remained at similar levels on Mustang Island, and increased on North Padre and Matagorda islands.

For each stranded turtle, information was collected on species, stranding date and location, tag numbers (if applicable), visible injuries, condition, and final disposition of the animal. The curved carapace length (CCL) and curved carapace width (CCW) or straight-line carapace length (SLCL) and straight-line carapace width (SLCW) of most turtles were measured. Information was recorded on standardized forms that were forwarded to the state and subsequently to the national STSSN coordinators.

The Texas STSSN database was queried for records of stranded turtles found in Texas from 1980 through 1994. Stranded turtles that had been raised in captivity for about one year (head-started) were excluded from this analysis because the temporal and spatial distributions of their strandings may have been influenced by captive rearing and release (Manzella et al., 1988; Teas, 1993). Similarly, turtles that were captured incidentally (such as in power plant intake canals and by commercial or recreational fishing) were not included in the total number of strandings (Teas, 1993).

Strandings were categorized as either offshore (on beaches or waters of the Gulf of Mexico) or inshore (in the passes and bays) (Teas, 1993). The locations of offshore and inshore strandings were also categorized by year and by Shrimp Statistical Zone, established by the National Marine Fisheries Service. Strandings were also grouped according to whether they occurred along the upper Texas coast (Zones 17 and 18) (Caillouet et al., 1991), middle Texas coast (Zone 19), or the lower Texas coast (Zones 20 and 21) (Fig. 1).

Injuries and anomalous conditions of the turtles that possibly contributed to the strandings were summarized from comments on stranding forms. Turtles were categorized as hatchlings/post-hatchlings when they were less than 10.0 cm SLCL and as adults when they exceeded minimum sizes of nesting females. Conversions of minimum nesting sizes to curved measurements were made with regression equations developed by Teas (1993). Kemp's ridley turtles larger than 58.8 cm SLCL (Marquez-M, 1994) or 62.1 cm CCL [derived with Teas (1993)], loggerhead turtles larger than 90.0 cm SLCL (Teas[1]) or 96.5 cm CCL [derived with Teas (1993)], and leatherback turtles larger than 125.0 cm SLCL (Marquez-M, 1990) were classified as adults. Turtles were considered juveniles or sub-adults when they were larger than hatchlings/post-hatchlings but smaller than adults. The sexes of adults were determined during necropsies.

[1] Teas, Wendy. 1994. Miami Laboratory, Southeast Fisheries Science Center, NMFS, 75 Virginia Beach Drive, Miami, FL 33149. Personal commun.

Necropsies and Digestive Tract Contents, 1994

Live stranded turtles were taken to various rehabilitation facilities in Texas. From 1980 through 1993, some of the dead stranded turtles were salvaged for later necropsy or use in conjunction with foraging ecology studies (Heinly et al., 1988; Shaver, 1991; Plotkin et al., 1993), and the remaining turtles were either marked, buried, or pulled behind the dunes. During 1994, only dead turtles that were extremely decomposed or found in remote locations were marked, buried, or pulled behind the dunes. All turtles that died during rehabilitation and most of the dead stranded turtles that were not highly deteriorated were salvaged for necropsy and study during 1994.

General necropsies, similar to those described by Wolke and George (1981), were performed by a limited number of STSSN participants and veterinarians. During necropsy, the sex of the turtle was determined by visual examination of the gonads. Most necropsies of the turtles that stranded during 1994 were conducted either at the National Marine Fisheries Service Laboratory in Galveston, Texas, or at the Padre Island National Seashore in Corpus Christi, Texas.

At Padre Island, I necropsied 142 stranded turtles found along the lower Texas coast during 1994, including 59 loggerhead, 47 Kemp's ridley, 31 green, 4 hawksbill, and 1 leatherback turtle. When possible, I removed the entire digestive tract from each turtle. The contents were either frozen for later examination or immediately examined. The items ingested by the 37 Kemp's ridley turtles were identified, categorized into seven general food groups, baked in a drying oven, and weighed according to procedures outlined by Shaver (1991).

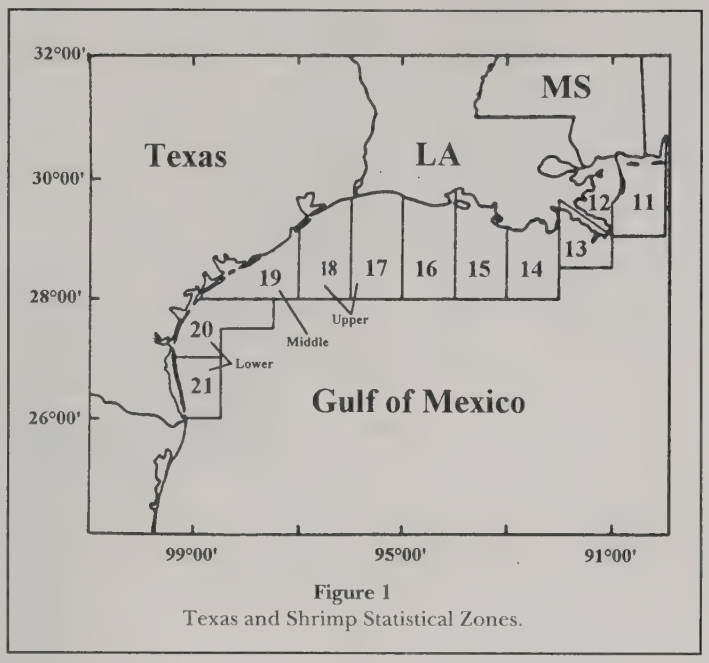


Figure 1
Texas and Shrimp Statistical Zones.

Statistical Analyses

Data were subjected to the Kolmogorov-Smirnov normality test and Bartlett's test for homogeneity of variances before statistical analyses were performed. In all analyses, differences were considered significant at $P<0.05$. SIGMASTAT (1992) statistical software package was used for all statistical procedures. Means are followed by ± one standard error.

The annual numbers of stranded turtles located during the Texas Closure in 1981–94 were compared with the annual numbers of stranded turtles located during the same length of time, before and after the closure, in those years. Because the numbers of turtles found stranded before, during, and after the Texas Closure were not normally distributed ($P<0.001$), non-parametric Kruskal-Wallis one way analyses of variance on ranks were used to compare the median number of turtles in each group and Student-Newman-Keuls all pairwise multiple comparisons tests were used to isolate which groups differed from the others. Tests were repeated excluding years when large numbers of turtles stranded as a result of identified sources other than incidental capture in shrimp trawls.

I used *t*-tests to compare the mean numbers of stranded turtles found per day in offshore and inshore waters and during closure and non-closure periods in 1981–94, and non-parametric Mann-Whitney rank sum tests to compare the median values of the groups when the assumptions for parametric tests (normality and equal variance) were violated. I used *t*-tests and Mann-Whitney rank sum tests to compare the percentages of stranded turtles that were located in offshore and inshore areas during closure and non-closure periods in 1981–94. Both set of tests were repeated excluding years when large numbers of turtles stranded as a result of identified sources other than incidental capture in shrimp trawls.

SLCL's of stranded Kemp's ridley turtles found along the upper, middle, and lower Texas coast between 1980 and 1994 were compared. SLCL's of individuals for which only CCL's were available were derived with Teas (1993). Because Kemp's ridley SLCL's were not normally distributed ($P\leq0.05$), non-parametric Kruskal-

Wallis one way analyses of variance on ranks were used to compare median SLCL's of each group and Dunn's method all pairwise multiple comparisons tests to isolate which groups differed from the others. Mann-Whitney rank sum tests were used to compare median SLCL's of Kemp's ridley turtles stranded inshore and offshore along the upper, middle, and lower Texas coast.

SLCL's of all species stranded during 1994 were compared using non-parametric tests since SLCL's for each species were not normally distributed ($P<0.05$). A Kruskal-Wallis one way analysis of variance on ranks was used to compare median SLCL's among the species and a Dunn's method all pairwise comparisons test was used to isolate which species differed from the others.

I compared the SLCL's, digestive tract content weights, percent dry masses of crabs, and percent dry masses of fishes in the digestive tracts of 37 wild Kemp's ridley turtles that stranded in 1994 and 50 that stranded from 1983 to 1989 (Shaver, 1991). Because all four of the variables failed normality tests ($P<0.001$), I used non-parametric Mann-Whitney rank sum tests for the comparisons.

Results

Strandings, 1980–94

From 1980 to 1994, 3,283 stranded sea turtles, including 1,139 (34.7%) Kemp's ridley, 1,524 (46.4%) loggerhead, 258 (7.9%) green, 170 (5.2%) hawksbill, 57 (1.7%) leatherback, and 135 (4.1%) unidentified turtles, were located along the Texas coast. An additional 252 head-started Kemp's ridley and 10 head-started loggerhead turtles were documented but excluded from further analyses.

Three hundred fifty-four (10.8%) of the 3,283 stranded turtles were alive and 2,929 (89.2%) were dead when located. Species composition varied by year; Kemp's ridley turtles comprised an increasing percentage of the strandings throughout time (Fig. 2A). The most abundant species found during February was the green turtle whereas Kemp's ridley and loggerhead turtles predominated during other months of the year (Fig. 3A).

Of the 3,283 stranded turtles, 2,876 (87.6%) were located in offshore, 401 (12.2%) in inshore, and 6 (0.2%) in unknown areas (Figs. 2C, 3C). Species composition varied in offshore and inshore areas. The 2,876 turtles found in offshore areas included 982 (34.1%) Kemp's ridley, 1,451 (50.5%) loggerhead, 124 (4.3%) green, 155 (5.4%) hawksbill, 54 (1.9%) leatherback, and 110 (3.8%) unidentified turtles. The 401 found inshore included 156 (38.9%) Kemp's ridley, 72 (18.0%) loggerhead, 132 (32.9%) green, 13 (3.3%) hawksbill, 3 (0.7%) leatherback, and 25 (6.2%) unidentified turtles.

Most strandings occurred between March and November (Fig. 3C). Strandings decreased during June. During 1981–94, with and without the excluded years, the numbers of stranded turtles located during the Texas Closure were significantly different from the numbers located during the same length of time, before and after the closure (Table 1). Fewer turtles were located during the closure than before and after it. Additionally, fewer were located after the closure than before it. However, the numbers of stranded turtles located in inshore areas during the Texas Closure were not significantly different from the numbers located in inshore areas during the same length of time, before and after the closure (Table 1).

During 1981–94, with and without the excluded years, fewer stranded turtles were found per day during the Texas Closure than during the remainder of the year, when Gulf of Mexico waters were open to shrimp trawling (Table 1). Fewer stranded turtles were found per day in offshore areas during the Texas Closure than in offshore areas during the remainder of the year. However, the number of stranded turtles found per day in inshore areas during the Texas Closure and during the remainder of the year did not differ.

Fewer stranded turtles were found per day in inshore areas than in offshore areas when Gulf waters were open to shrimping, during 1981–94 (Mann-Whitney, $T=105$, $N(\text{small})=14$, $N(\text{big})=14$, $P<0.001$) and during 1981–94 with years 1986, 1989, and 1990 excluded (Mann-Whitney, $T=66$, $N(\text{small})=11$, $N(\text{big})=11$, $P<0.001$). Similarly, fewer turtles were found stranded per day in inshore areas than in offshore areas during the Texas Closure from 1981–94 (t-test, $t=-4.012$, df=26, $P<0.001$) and during 1981–94 with years 1986, 1989, and 1990 excluded (t-test, $t=-3.609$, df=20, $P=0.002$).

The percentage of stranded turtles located in offshore areas during the Texas Closure was not significantly different from the percentage located in offshore areas during the remainder of the year during 1981–94 (Mann-Whitney, $T=189$, $N(\text{small})=14$, $N(\text{big})=14$, $P=0.534$) and during 1981–94 with years 1986, 1989, and 1990 excluded (Mann-Whitney, $T=125$, $N(\text{small})=11$, $N(\text{big})=11$, $P=0.947$). However, during 1990–94 (the only years when all Gulf of Mexico waters off Texas were closed to shrimp trawling out to 322 km) the percentage of stranded turtles located in offshore areas during the Texas Closure was smaller than the percentage located in offshore areas during the remainder of the year (t-test, $t=4.261$, df=8, $P=0.003$).

The percentage of strandings in inshore areas was greatest during January, February, and June (Fig. 3C). The percentage of stranded turtles located in inshore areas during the Texas Closure was not significantly different from the percentage located in inshore areas during the remainder of the year for 1981–94 (Mann-

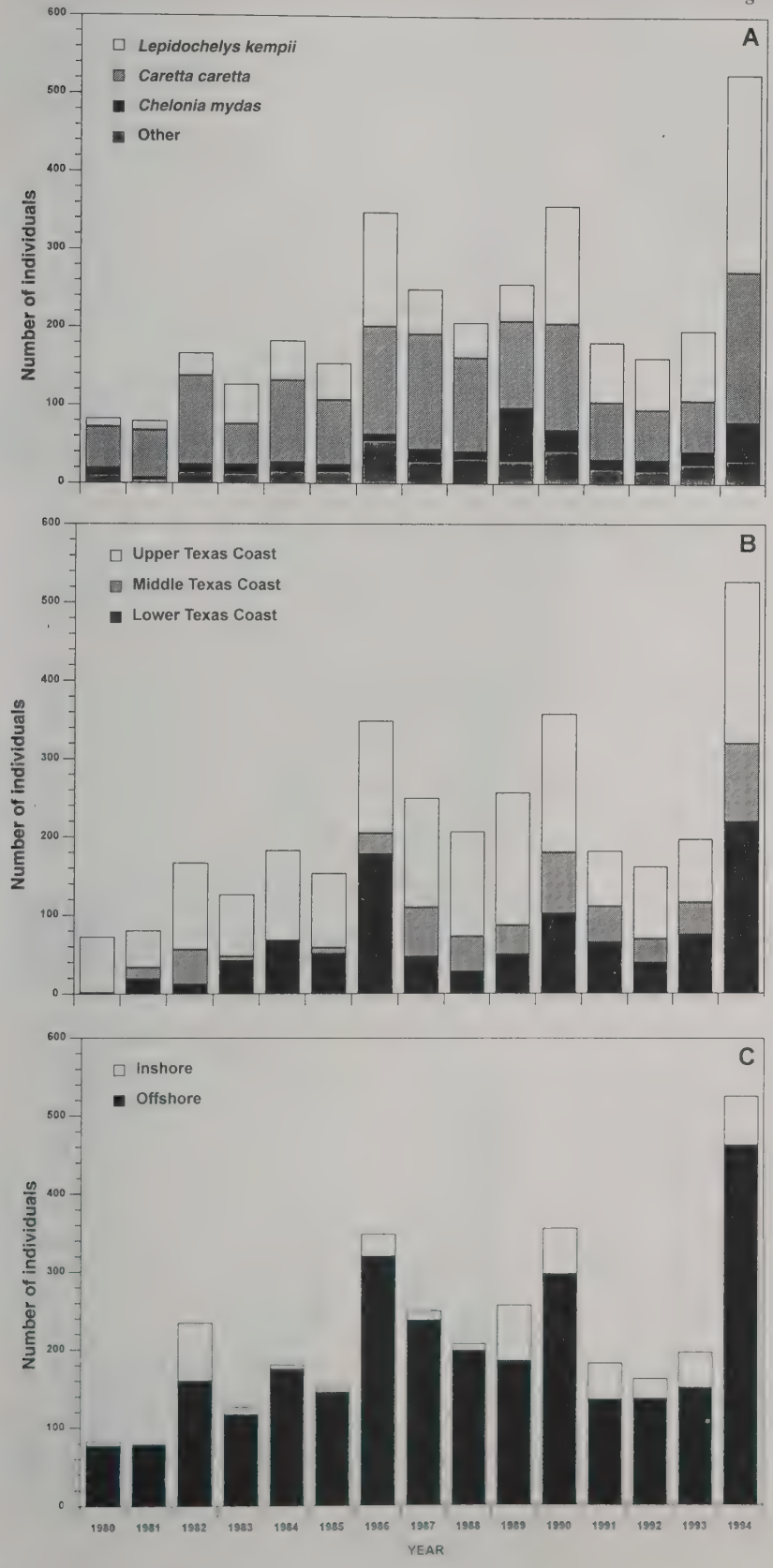
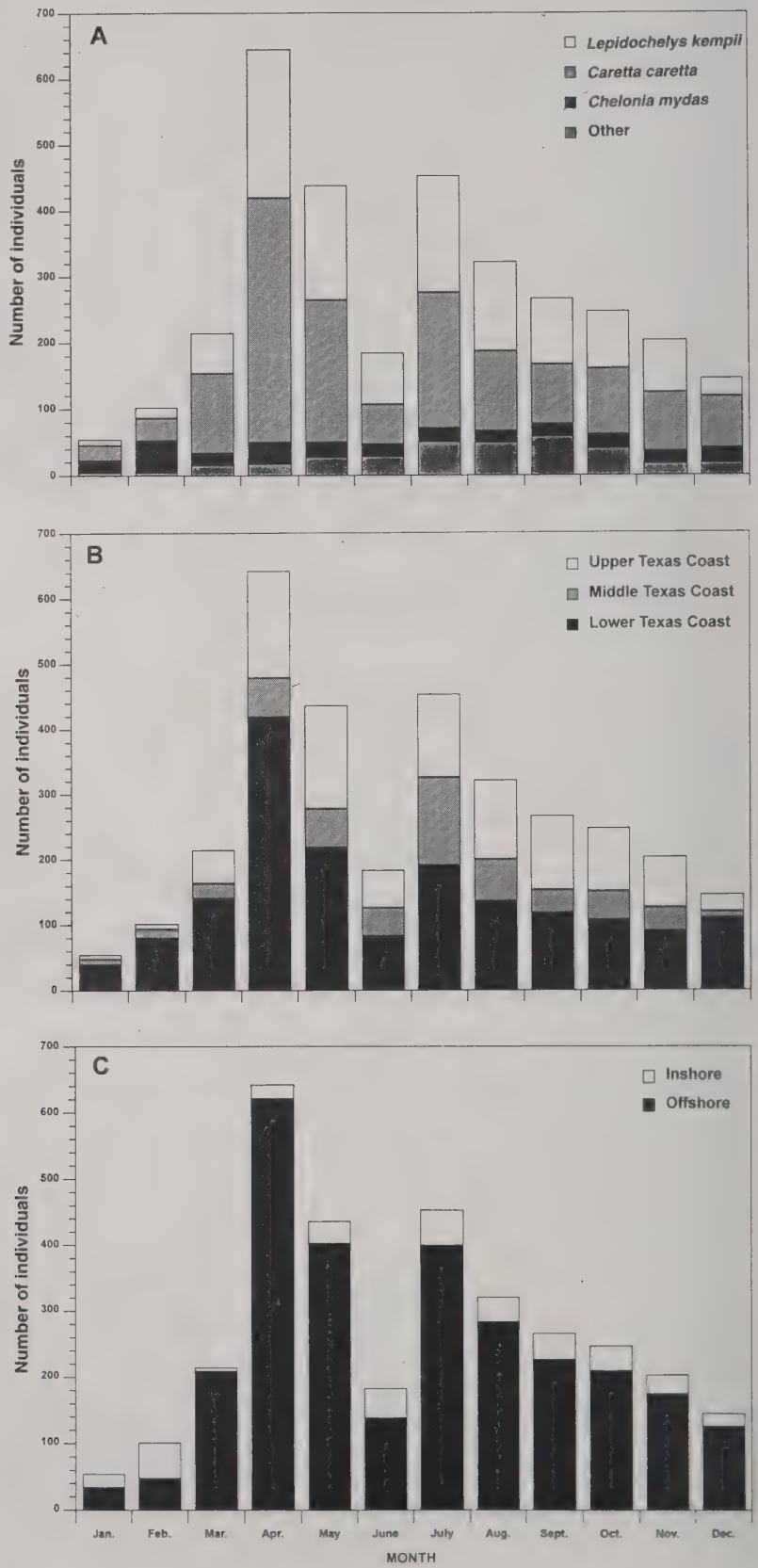


Figure 2

Annual number of stranded sea turtles found along the Texas coast from 1980–94 by (A) species, (B) region, and (C) area. Species include Kemp's ridley, *Lepidochelys kempii*, loggerhead, *Caretta caretta*, green, *Chelonia mydas*, and other (hawksbill, *Eretmochelys imbricata*, leatherback, *Dermochelys coriacea*, and unidentified species turtles combined). Regions include upper Texas coast (Zones 17, 18), middle Texas coast (Zone 19), and lower Texas coast (Zones 20, 21). Areas include inshore and offshore.

Figure 3

Monthly number of stranded sea turtles found along the Texas coast from 1980–94 by (A) species, (B) region, and (C) area. Species include Kemp's ridley, *Lepidochelys kempii*, loggerhead, *Caretta caretta*, green, *Chelonia mydas*, and other (hawksbill, *Eretmochelys imbricata*, leatherback, *Dermochelys coriacea*, and unidentified species turtles combined). Regions include upper Texas coast (Zones 17, 18), middle Texas coast (Zone 19), and lower Texas coast (Zones 20, 21). Areas include inshore and offshore.



Whitney, T=189, N(small)=14, N(big)=14, P=0.534) and for 1981–94 with years 1986, 1989, and 1990 excluded (Mann-Whitney, T=125, N(small)=11, N(big)=11, P=0.947). However, inshore strandings comprised an increasing percentage of the strandings, particularly during the Texas Closure, throughout time (Figs. 2C, 3C). Additionally, during 1990–94, the percentage of stranded turtles located in inshore areas during the Texas Closure was larger than the percentage located in inshore areas during the remainder of the year (t-test, t=–4.621, df=8, P=0.002).

Strandings were distributed throughout the state; 52 (1.6%) were in Zone 17, 939 (28.6%) in Zone 18, 548 (16.7%) in Zone 19, 1,327 (40.4%) in Zone 20, 415 (12.6%) in Zone 21, and 2 (0.1%) in unknown areas (Figs. 2B, 3B). Grouping strandings into regions of the Texas coast, 991 (30.2%) occurred on the upper Texas coast, 548 (16.7%) on the middle Texas coast, and 1,742 (53.1%) on the lower Texas coast. Strandings per region varied for different years and months (Figs. 2B, 3B).

More stranded Kemp's ridley turtles were located along the upper Texas coast (613), than the middle

Table 1

Test 1: Kruskal-Wallis one way ANOVA on ranks to compare the number of stranded turtles found during the Texas Closure (DC) versus the number found during the same number of days before the closure (BC) and after the closure (AC), followed by Student-Newman-Keuls method all pairwise multiple comparisons to isolate which groups differ. Test 2: Mann-Whitney rank sum tests to compare the number of stranded turtles found per day during the Texas Closure (C) versus the number found per day during the remainder of the year (O). Median numbers found during BC, DC, AC, C, and O are in parentheses. * designates significant difference, P<0.05.

	Test 1			Test 2		
Areas and years	Test statistic	df	P	Test statistic	N(small), N(big)	P
All waters, 1981–94	H=16.43	2	<0.001 *	T=145	14, 14	0.008 *
BC (52.0) vs. DC (18.5) Test 1	q=5.708		<0.05 *			
DC (18.5) vs. AC (42.5) Test 1	q=4.938		<0.05 *			
BC (52.0) vs. AC (42.5) Test 1	q=3.574		<0.05 *			
C (0.390) vs. O (0.555) Test 2[1]						
All waters, 1981–94 (exclusions)[2]	H=14.05	2	<0.001 *	T=91	11, 11	0.021 *
BC (47.5) vs. DC (18.0) Test 1	q=5.288		<0.05 *			
DC (18.0) vs. AC (36.5) Test 1	q=4.368		<0.05 *			
BC (47.5) vs. AC (36.5) Test 1	q=3.511		<0.05 *			
C (0.380) vs. O (0.520) Test 2						
Offshore waters, 1981–94	H=20.883	2	<0.001 *	T=142	14, 14	0.005 *
BC (50.0) vs. DC (13.5) Test 1	q=6.449		<0.05 *			
DC (13.5) vs. AC (33.5) Test 1	q=5.247		<0.05 *			
BC (50.0) vs. AC (33.5) Test 1	q=4.370		<0.05 *			
C (0.245) vs. O (0.485) Test 2						
Offshore waters, 1981–94 (exclusions)[2]	H=17.871	2	<0.001 *	T=92	11, 11	0.025
BC (47.5) vs. DC (12.5) Test 1	q=5.973		<0.05 *			
DC (12.5) vs. AC (29.5) Test 1	q=4.572		<0.05 *			
BC (47.5) vs. AC (29.5) Test 1	q=4.327		<0.05 *			
C (0.240) vs. O (0.430) Test 2						
Inshore waters, 1981–94	H=0.464	2	0.793	T=198	14, 14	0.835
C (0.050) vs. O (0.060) Test 2						
Inshore waters, 1981–94 (exclusion)[3]	—	—	—	T=178	13, 13	0.918
C (0.040) vs. O (0.030) Test 2						

[1] Results for each Test 2 are presented across from the preceding group heading.

[2] For Test 1, years 1986 and 1990 were excluded because large numbers of turtles stranded in offshore waters as the result of identified sources other than incidental capture in shrimp trawls (see Discussion) during the comparison dates. For Test 2, years 1986 and 1990 were excluded because of the preceding reasons and 1989 was excluded because large numbers of turtles stranded in inshore waters as the result of identified sources other than incidental capture in shrimp trawls, during dates not encompassed in Test 1.

[3] Test 1 was not conducted because no exclusions were necessary (no large numbers of turtles stranded in inshore waters as the result of identified sources other than incidental capture in shrimp trawls during the comparison dates). For Test 2, year 1989 was excluded because large numbers of turtles stranded in inshore waters as the result of identified sources other than incidental capture in shrimp trawls, during dates not encompassed in Test 1.

Table 2

Straight-line carapace lengths (cm) of stranded Kemp's ridley turtles, *Lepidochelys kempii*, found on the Texas coast during 1980–94. Statistical comparisons of the lengths of these individuals are summarized in Table 3.

	All individuals			No individuals < 10.0 cm SLCL		
Area	Mean	(N, SE)	Code[1]	Mean	(N, SE)	Code
Overall (offshore and inshore areas)			A			D
Upper Texas coast	37.4	(527, 0.5)	1	37.5	(525, 0.5)	10
Middle Texas coast	47.6	(151, 1.2)	2	48.2	(149, 1.2)	11
Lower Texas coast	38.2	(317, 1.2)	3	48.0	(244, 0.9)	12
Offshore waters			B			E
Upper Texas coast	37.5	(493, 0.6)	4	37.6	(491, 0.6)	13
Middle Texas coast	51.6	(81, 1.6)	5	52.8	(79, 1.4)	14
Lower Texas coast	37.8	(294, 1.3)	6	48.6	(221, 1.0)	15
Inshore waters			C			F
Upper Texas coast	35.8	(33, 1.8)	7	35.8	(33, 1.8)	16
Middle Texas coast	43.0	(70, 1.7)	8	43.0	(70, 1.7)	17
Lower Texas coast	43.1	(23, 3.0)	9	43.1	(23, 3.0)	18

[1] Each letter code designates a subset of the strandings that is comprised of the three geographical areas, designated by number codes, listed below it.

(185) or lower (341) Texas coast. Although more Kemp's ridleys were located stranded in offshore areas along the upper Texas coast (570) than in offshore areas along the middle (100) and lower (312) Texas coast, more were found in inshore areas along the middle Texas coast (85) than the upper (42) and lower (29) Texas coast.

The size of stranded Kemp's ridley turtles varied in the state (Tables 2, 3). The smallest individuals, hatchlings/post-hatchlings, were located almost exclusively along the lower Texas coast. From 1980 to 1994, 73 were found on the lower Texas coast, 2 on the upper Texas coast, and 2 on the middle Texas coast (Fig. 4). Stranded Kemp's ridley turtles located in offshore areas and overall (offshore and inshore areas collectively) along the middle Texas coast were significantly larger than those located in the areas along the upper and lower Texas coast (Tables 2, 3). However, with hatchlings and post-hatchlings excluded, Kemp's ridley turtles from the middle and lower Texas coast were significantly larger than those located along the upper Texas coast. Stranded Kemp's ridley turtles found in inshore areas along the middle Texas coast were significantly smaller than those found in the offshore areas. However, those found in inshore areas along the upper and lower Texas

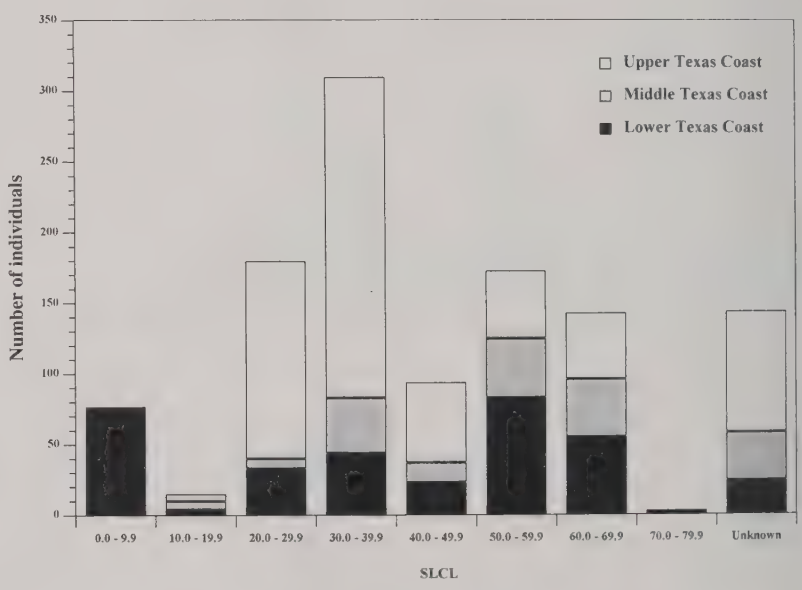


Figure 4

Straight-line carapace lengths (SLCL's) of stranded Kemp's ridley turtles, *Lepidochelys kempii*, found on the Texas coast from 1980–94 (*N*=1,139).

Table 3

Kruskal-Wallis one way ANOVA on ranks, Dunn's method all pairwise multiple comparisons, and Mann-Whitney rank sum tests used to compare median straight-line carapace lengths of stranded Kemp's ridley turtles found on the Texas coast from 1980–94; * designates significant difference, $P<0.05$. SLCL group codes are presented in Table 2.

Codes	Test	Test statistic	df or N	P
A	Kruskal-Wallis	H=51.611	df=2	<0.001*
B	Kruskal-Wallis	H=51.268	df=2	<0.001*
C	Kruskal-Wallis	H=8.572	df=2	0.014*
D	Kruskal-Wallis	H=111.700	df=2	<0.001*
E	Kruskal-Wallis	H=116.600	df=2	<0.001*
F	Kruskal-Wallis	H=8.572	df=2	0.014*
1 vs. 2	Dunn's method	Q=7.184		<0.05*
2 vs. 3	Dunn's method	Q=5.156		<0.05*
1 vs. 3	Dunn's method	Q=2.156		>0.05
4 vs. 5	Dunn's method	Q=7.160		<0.05*
5 vs. 6	Dunn's method	Q=5.917		<0.05*
4 vs. 6	Dunn's method	Q=1.572		>0.05
7 vs. 9	Dunn's method	Q=2.276		>0.05
10 vs. 11	Dunn's method	Q=8.000		<0.05*
11 vs. 12	Dunn's method	Q=0.602		>0.05
10 vs. 12	Dunn's method	Q=8.777		<0.05*
13 vs. 14	Dunn's method	Q=8.232		<0.05*
14 vs. 15	Dunn's method	Q=2.334		>0.05
13 vs. 15	Dunn's method	Q=8.543		<0.05*
16 vs. 18	Dunn's method	Q=2.276		>0.05
4 vs. 7	Mann-Whitney	T=8419	N=33, N=493	0.744
5 vs. 8	Mann-Whitney	T=4417	N=70, N=81	<0.001*
6 vs. 9	Mann-Whitney	T=3953	N=23, N=294	0.485
13 vs. 16	Mann-Whitney	T=8353	N=33, N=491	0.714
14 vs. 17	Mann-Whitney	T=4277	N=70, N=79	<0.001*
15 vs. 18	Mann-Whitney	T=2274	N=23, N=221	0.092

Table 4

Stranded sea turtles found on the Texas coast during 1994.

Species	Jan.	Feb.	Mar.	Apr.	May 1–14	May 15–31	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Lepidochelys kempii (Kemp's ridley turtle)	0	2	1	54	64	12	19	32	35	10	11	11	3	254
Caretta caretta (Loggerhead turtle)	3	1	5	41	15	9	8	57	23	11	6	9	6	194
Chelonia mydas (Green turtle)	0	0	2	8	3	3	3	8	2	5	3	7	4	48
Eretmochelys imbricata (Hawksbill turtle)	0	0	0	0	1	0	2	1	2	7	0	0	1	14
Dermochelys coriacea (Leatherback turtle)	0	0	0	1	2	0	0	0	0	0	0	0	0	3
Unknown	0	0	0	1	1	2	2	3	2	2	0	0	1	14
Total	3	3	8	105	86	26	34	101	64	35	20	27	15	527

coast were only slightly smaller than those found in offshore areas there.

Strandings, 1994

Along the Texas coast, 527 wild sea turtles were found stranded during 1994 (Table 4), only 14 fewer than the number stranded during the years 1991, 1992, and 1993 combined (541) and more than during any previous year on record for the Texas STSSN (Fig. 2A–C). Previous yearly totals ranged from 83 in 1980 to 358 in 1990. During 1994, monthly stranding totals for April, May, June, July, and August (Table 4) exceeded numbers previously documented during those months for all years of record. Although 33 head-started Kemp's ridley turtles and 2 head-started loggerhead turtles were also documented stranded during 1994, these turtles

were not included in stranding totals and analyses presented herein.

Of the 527 strandings, 219 (41.5%) were located on the upper Texas coast, 101 (19.2%) on the middle Texas coast, and 207 (39.3%) on the lower Texas coast (Fig. 5A). For those located in the upper Texas coast, 13 were from Zone 17 and 206 from Zone 18. Lower Texas coast strandings included 157 from Zone 20 and 50 from Zone 21.

Strandings increased dramatically during early April (week 14) and continued at high levels through mid May (week 19) (Fig. 5A, B). From April through mid May, strandings were concentrated on Galveston (upper Texas coast), Mustang (lower Texas coast), and North Padre (lower Texas coast) islands. Increases in strandings were first detected along the lower Texas coast in early April. About one week later, increases were also detected along the upper Texas coast. Although strandings decreased along the lower Texas coast by early May, they continued at high levels along the upper Texas coast until 14 May. Strandings abruptly decreased and remained at relatively low levels throughout the state until 10 July (week 28), when another peak was detected. Strandings during the July peak were concentrated in the Galveston, Matagorda (middle Texas coast), and Mustang island areas. Although strandings decreased during late July (week 30), another peak occurred during late August (weeks 34–35). Most of the turtles documented during the August peak were found in the vicinity of Galveston Island. Strandings remained at relatively low levels during the last 4 months of the year (weeks 36–52).

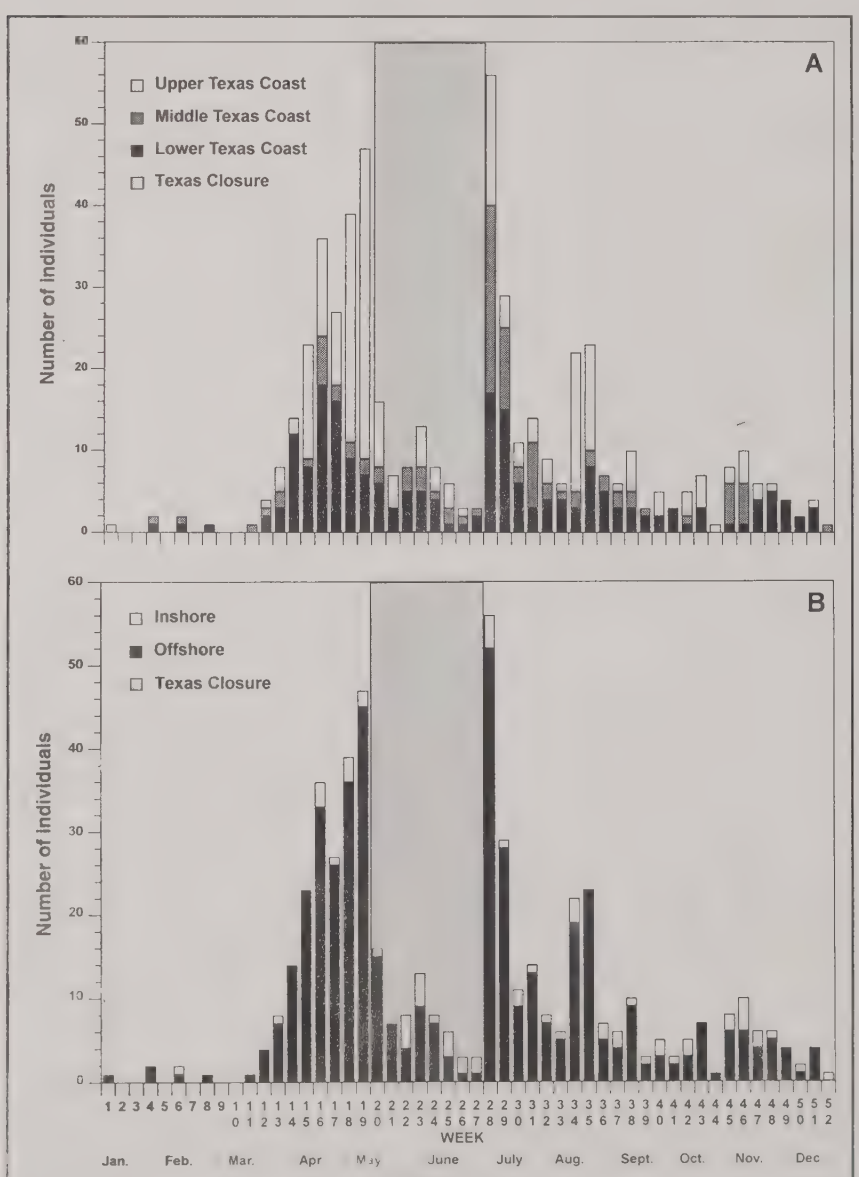


Figure 5

Weekly number of stranded sea turtles found on the Texas coast during 1994 by (A) region and (B) area. Regions include upper Texas coast (Zones 17, 18), middle Texas coast (Zone 19), and lower Texas coast (Zones 20, 21). Areas include inshore and offshore.

Of the 527 strandings, 463 were documented from offshore areas, 63 from inshore areas, and 1 from an unknown area (Fig. 5B). From 15 May through 9 July (weeks 20–27), 27% of the strandings were documented from inshore areas, compared with only 10% from inshore areas during other times of the year.

Species composition of the 527 stranded turtles included 254 Kemp's ridley, 194 loggerhead, 48 green, 14 hawksbill, 3 leatherback, and 14 of unknown species (Table 4). Forty-eight percent of the turtles found were Kemp's ridley, the highest percentage of this species recorded for Texas in STSSN history (Fig. 2A).

Table 5
Straight-line carapace lengths (SLCL's) (cm) of stranded sea turtles found on the Texas coast during 1994.

Species	Mean	N	SE	Median	Range	Code[1]
Lepidochelys kempii (Kemp's ridley turtle)	39.0	192	0.9	35.2	4.5–66.6	1
Caretta caretta (Loggerhead turtle)	65.7	157	1.2	66.1	5.2–101.3	2
Chelonia mydas (Green turtle)	32.4	38	1.2	30.3	23.7–60.9	3
Eretmochelys imbricata (Hawksbill turtle)	12.8	11	2.2	9.7	5.3–26.2	4
Dermochelys coriacea (Leatherback turtle)	—	2	—	—	134.2–138.0	—

[1] *designates significant difference, $P<0.05$. Median SLCL's of species (codes 1–4) are different (Kruskal-Wallis one way ANOVA on ranks, $H=217.400$, df=3, $P<0.001$*). Dunn's method all pairwise multiple comparison: 1 vs. 2, $Q=12.408$, $P<0.05$*; 2 vs. 3, $Q=9.642$, $P<0.05$*; 2 vs. 4, $Q=8.013$, $P<0.05$*; 1 vs. 4, $Q=3.731$, $P<0.05$*; 1 vs. 3, $Q=2.269$, $P>0.05$; 3 vs. 4, $Q=2.202$, $P>0.05$.

Of the 527 stranded turtles, 490 were found dead and 37 alive. The 37 live stranded turtles included 7 Kemp's ridley, 5 loggerhead, 14 green, 10 hawksbill, and 1 unidentified species. Only 3% of the stranded Kemp's ridley and loggerhead turtles were found alive. However, 29% of the green and 71% of the hawksbill turtle strandings were of live individuals. Of the 37 turtles found alive and taken to various rehabilitation facilities in Texas, 15 died, 11 were rehabilitated and released, and 11 were held for prolonged rehabilitation.

Most of the live Kemp's ridley and loggerhead (9 of 12) died during rehabilitation efforts, whereas most live green and hawksbill turtles (18 of 24) were successfully rehabilitated and released or are still being held. Most of the stranded Kemp's ridley turtles were sub-adults (Table 5, Fig. 6). Other Kemp's ridley turtles found included 29 adults (including five males, nine females, and 15 of unknown gender), four hatchlings/post-hatchlings, and a few juveniles. Among the nine adult females, four possessed tags that were placed on them at the nesting beach in Rancho Nuevo, Mexico, and one contained 39 eggs. Similarly, most loggerhead turtles were sub-adults. However, nine adults (including one male, one female, and seven of unknown gender), two hatchlings/post-hatchlings, and a few juveniles were also found. Green turtles found were juveniles and sub-adults. Seven of the stranded hawksbills were post-hatchlings, and the other seven were juveniles. Both leatherbacks measured were adults (including one female and one of unknown gender). Stranded loggerhead turtles were significantly larger than stranded Kemp's ridley, green, and hawksbill turtles (Table 5).

According to STSSN forms, four of the stranded turtles had ingested hooks, four were found alive lodged in rocks, 24 had boat propeller injuries, 13 were entangled in marine debris, 12 had been bitten by sharks, and 20 had straight-edged cuts at the bases of missing appendages, typical of human-inflicted mutilation (Heinly et al., 1988). In most instances the STSSN participant could not determine whether the bites and mutilation occurred before or after death. Two dead green turtles were found in abandoned illegal gill nets offshore from the southern end of Padre Island National Seashore. Eleven turtles were found with water coming out of the mouth or froth in the trachea.

Necropsies have been completed for all of the stranded sea turtles that were salvaged during 1994. The deteriorated condition of most of the salvaged turtles prohibited conclusive determination of the cause of death in most instances. Although marine debris was found in several of the turtles necropsied, in most instances the amounts were minimal and were probably not the cause of stranding or death. Only a few of the turtles found stranded were apparently ill for an extended period prior to their demise, as evidenced by emaciation, encrustation with epizoans, large quantities of internal parasites, or lack of appreciable quantities of ingested food items.

Kemp's Ridley Digestive Tract Content Analyses

None of the 37 wild Kemp's ridley turtles analyzed for digestive tract contents from 1994 appeared to have been ill prior to death. Gut contents were present in all individuals analyzed, indicating recent foraging. Gut content weights from the 1994 Kemp's ridley turtles were significantly greater than weights from the 1983–89 Kemp's ridley turtles (Mann-Whitney, $T=1900$, $N(\text{small})=37$, $N(\text{big})=50$, $P=0.020$), even though there was no significant difference between SLCL's of the two groupings (Mann-Whitney, $T=1744$, $N(\text{small})=36$, $N(\text{big})=50$, $P=0.120$).

Turtles from the 1983–89 and 1994 groupings had similar percent dry mass of the seven food categories (Table 6). Crabs composed 95% of the dry mass in both groups of ridleys. There was no significant difference between the percent dry mass of crabs between the two groups (Mann-Whitney, $T=1716$, $N(\text{small})=37$, $N(\text{big})=50$, $P=0.451$). Fish were consumed by 24% of the 1994

ridleys compared with 14% of those from 1983–89. However, fishes composed less than 1% of the total gut content mass for the 1983–89 and 1994 Kemp's ridley turtles (Table 6), and no significant difference was found in the percent dry mass of fishes between the two groups (Mann-Whitney, T=1742, N(small)=37, N(big)=50, P=0.313).

Although marine debris was ingested by 19% of the 1994 Kemp's ridleys compared with 34% by the 1983–89 group, marine debris composed less than 0.1% of the total gut content dry mass for the two groups (Table 6). Only minute amounts of debris were found, and in no instance did it appear that ingestion of marine debris led directly to the demise of the animal.

Table 6

Percent dry mass and percent frequency of food groups found in digestive tract contents of wild Kemp's ridley turtles, *Lepidochelys kempii*, from south Texas during 1983–89 (N=50; Shaver, 1991) and 1994 (N=37).

	Percent dry mass		Percent frequency	
Item	1983–89	1994	1983–89	1994
Crabs	95.22	95.56	80.00	83.78
Mollusks	1.56	1.62	58.00	89.19
Fishes	0.08	0.72	14.00	24.32
Vegetation	0.14	0.21	56.00	54.05
Shrimp	0.18	0.02	4.00	8.11
Other materials	2.74	1.82	72.00	67.57
Marine debris	0.08	0.05	34.00	18.92

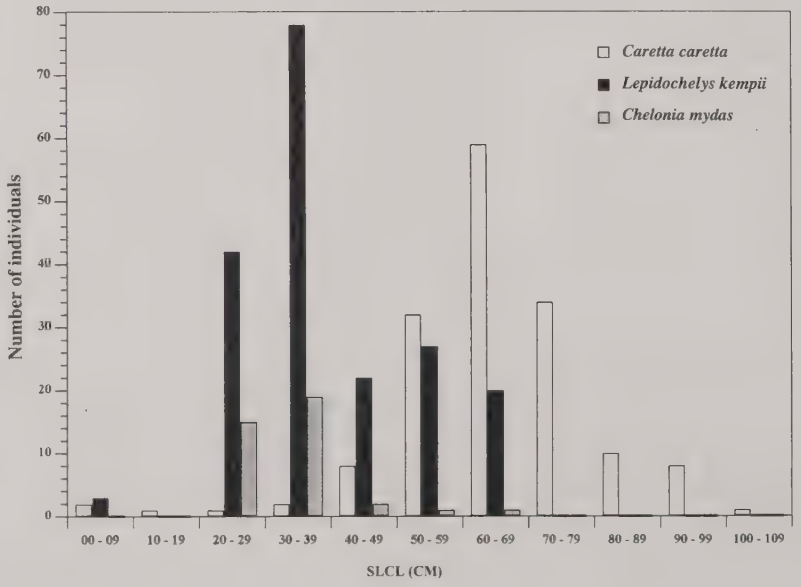


Figure 6

Straight-line carapace lengths of stranded loggerhead turtles, *Caretta caretta*, (N=153), Kemp's ridley turtles, *Lepidochelys kempii*, (N=192), and green turtles, *Chelonia mydas*, (N=38) found on the Texas coast during 1994.

Discussion

Prior to the adoption of regulations requiring the usage of TED's, correlations were found between shrimping effort and strandings of sea turtles in Texas (Whistler, 1989; Caillouet et al., 1991; Sis et al., 1993). Magnuson et al. (1990) estimated that 70–80% of the turtles stranded during the shrimping season in Texas were caught and killed in shrimp trawls and that as many as 44,000 turtles were killed annually by shrimp trawls in U.S. coastal waters of the Atlantic Ocean and the Gulf of Mexico. Magnuson et al. (1990) also concluded that incidental capture of sea turtles in shrimp trawls was the major cause of mortality associated with human activities and resulted in the death of more sea turtles than all other human activities combined.

Beginning in 1990, TED's were phased into mandatory usage, in an attempt to reduce trawl-related mortality. Documented turtle strandings in Texas decreased during 1991, 1992, and 1993. However, the pattern of higher numbers of strandings before and after the Texas Closure and reduced strandings during the closure (Magnuson et al., 1990) continued and more turtles were found stranded during 1994 than during any previous year on record for the Texas STSSN.

Comparisons of stranding numbers among years must be qualified. Varying effort was made to detect turtles between 1980 and 1994 and stranding totals for some years were skewed by events such as hatchling/post-hatchling strandings and hypothermic stunnings. When these conditions are considered, the relative number of turtles found stranded during 1994 is even more substantial.

Numbers detected during the early 1980's were probably low because the STSSN was not fully operational at that time (Whistler, 1989). The two pre-

vious years of most numerous strandings in Texas were 1986 (349) and 1990 (358). Systematic surveys to locate stranded turtles were most comprehensive during the period 1986–90. Additional, undetected turtles probably stranded on the Matagorda Peninsula and San Jose Island during 1994 because numerous strandings were documented in adjacent areas receiving more comprehensive STSSN coverage.

More hatchling and post-hatchling sea turtles were found stranded along the Texas coast during 1986 (25) and 1990 (53) than during any other years of the STSSN (Shaver, 1992). Forty-seven of the 257 turtles found stranded during 1989 were located during or shortly after periods of freezing temperatures and were probable victims of hypothermic stunning (Shaver, 1990a). In contrast, only 13 hatchling/post-hatchling turtles and no hypothermic-stunned turtles were found stranded during 1994.

Comments listed on STSSN forms revealed possible causes for strandings of 47 of the 527 turtles found during 1994, including boat propeller injuries (24), debris entanglement (13), hook ingestion (4), entanglement in abandoned illegal gill netting (2), and being lodged in rocks (4). Although 11 turtles were found with water coming out of the mouth and or froth in the trachea, this condition has been disputed as conclusive evidence of drowning. Based on necropsies, illness and marine debris ingestion probably caused stranding of relatively few of the 527 turtles. Thus, no readily discernable cause was identified for most strandings from the STSSN forms and necropsies.

Several other possible causes for the strandings were suggested and investigated but were dismissed because of lack of supportive evidence (Shaver, 1994b; Zimmerman[2]). Among the unlikely contributing factors proposed were seismic exploration, oil and gas platform removal, menhaden fishing, anoxia (low water oxygen), pollutants, toxic wastes, dinoflagellate blooms, and ingestion of fish killed by any of the preceding factors.

A large percentage of the 1994 strandings was probably due to incidental capture in shrimp trawls. Stranding patterns closely followed nearshore shrimping patterns (Zimmerman[2]). When shrimping activity increased along the Texas coast during April and boats were most numerous in nearshore waters off Galveston, Mustang, and North Padre islands, strandings were most numerous in those areas. Strandings and nearshore shrimping effort were relatively low for the Matagorda Island area during April and for the North Padre Island area from May through the remainder of the year.

Strandings greatly decreased from 13 May to 7 July, when Gulf waters were closed to shrimping activities out to 322 km for the Texas Closure. When shrimping resumed in Gulf waters, large numbers of turtles were found stranded. Strandings at that time were concentrated in the Galveston, Matagorda, and Mustang island areas, where nearshore shrimping effort was high.

The apparent relationship between strandings and TED enforcement activities also supports the theory that many of the 1994 strandings were due to shrimping activity. In the latter part of July (week 30), when intensive TED enforcement and education activities occurred and nearshore shrimping effort decreased, strandings decreased. However, strandings increased again during late August (weeks 34–35), when TED enforcement activities decreased because U.S. Coast Guard enforcement personnel were shifted to Florida to deal with the Cuban refugee crisis. Strandings immediately decreased when TED enforcement activities increased during early September (week 36). Enforcement activities continued during September and strandings remained at relatively low levels for the rest of the year.

Operational or installation problems with TED's probably resulted in a large number of the strandings during 1994. Although 95% of the shrimp vessels inspected during April 1994 had TED's present in their nets, operational problems detected with the TED's may have caused turtles to be retained (Zimmerman[2]). Installation or operational problems were noted in half of the vessels inspected during mid July (Zimmerman[2]). Turtles may also have stranded as a result of capture and retention in trawls equipped with TED's since these devices are only 97% effective at excluding sea turtles (Zimmerman[2]). Soft and bottom shooting hard TED's, used by a large percentage of the shrimp fleet during 1994, may have been even less effective at excluding turtles (Zimmerman[2]). Repeated capture and release from TED-equipped trawls, capture in trawls with intentionally disabled TED's, and capture in try nets (which are not required to have TED's) may also have contributed to the turtle strandings. The shift of shrimping effort to nearshore areas may have increased the number of encounters that turtles had with trawlers.

Of the 65 stranded turtles found during the Texas Closure, 48 were documented from offshore beaches and 17 from inshore beaches. Many of the 48 found on offshore beaches may have succumbed prior to the closure and then may have taken several days to wash ashore and be detected. Also, some may have stranded during the closure as a result of being weakened by capture and release from trawls prior to the closure.

Incidental capture in shrimp trawls probably resulted in a large proportion of the offshore and inshore strandings along the Texas coast from 1980 to 1994. Nearly 88% of the strandings during this time were

[2] Zimmerman, Roger. 1994. Galveston Laboratory, Southeast Fisheries Science Center, NMFS, 4700 Ave. U, Galveston, TX 77551. Personal commun.

located in offshore areas and a large number of those were probably due to incidental capture in shrimp trawls. From 1981 to 1994, fewer turtles were located (overall and in offshore areas) during the Texas Closure than before and after the closure. Perhaps fewer were located after the closure than before it because a large proportion of the resident and transient turtles died prior to the closure, leaving fewer available to strand later in the year. Fewer turtles were found stranded per day (overall and in offshore areas) during the Texas Closure than during the remainder of the year, when Gulf of Mexico waters were open to shrimp trawling, for the years 1981–94. Additionally, fewer stranded in offshore areas during June, the only full month of the Texas Closure, than during all other spring, summer, and fall months, even though turtles are of equal or greater abundance during June (Shaver, 1994a).

Inshore shrimp trawling probably resulted in inshore strandings. During the Texas Closure, shrimp trawling was permitted in inshore waters, where TED's were not mandatory until December 1994. During 1990–94, the only years that all Gulf of Mexico waters off Texas were closed to shrimp trawling out to 322 km, inshore strandings comprised 35% of all strandings during the Texas Closure. Also during these years, the percentage of strandings that were located inshore was higher during the Texas Closure than during the remainder of the year. During 1994, 27% of the inshore strandings were found during the 8-week Texas Closure; 27% of the strandings during the closure were located in inshore areas compared with only 10% in inshore areas during other times of the year.

It must be noted that several other factors were identified as causing sea turtles to strand in Texas from 1980 to 1994 (Gitschlag and Renaud, 1989; Whistler, 1989; Plotkin and Amos, 1990; Shaver, 1990a; Shaver, 1990b; Caillouet et al., 1991; Shaver, 1992). Collision with boat propellers and hypothermic stunning resulted in numerous strandings and continue to be significant threats to sea turtles in Texas inshore areas.

From 1980–94, sea turtle strandings were distributed throughout the state, with 991 found along the upper Texas coast, 548 along the middle Texas coast, and 1,742 along the lower Texas coast. This stranding pattern may reflect greater turtle abundance or mortality along the lower Texas coast. Alternatively, it may reflect the variability in detection efforts for the STSSN. During the early 1980's, efforts to detect stranded turtles were probably greater along the lower Texas coast because the STSSN Texas coordinator was based out of that location. The majority of the middle Texas coast received little systematic coverage to document stranded turtles (except during 1986–89). However, a large portion of the lower Texas coast also received limited coverage. Lastly, the differences in stranding numbers could reflect differences in offshore beach length between the three regions. Although offshore beach length is nearly equal for the middle and lower Texas coast, it is approximately one-third smaller for the upper Texas coast.

Only 12% of the strandings were located in inshore areas from 1980 to 1994. However, inshore areas were not systematically surveyed for stranded turtles and some individuals probably were not detected. Inshore strandings comprised an increasing percentage of the overall strandings, perhaps reflecting increased abundance or mortality of turtles in inshore areas, or increased reporting of sea turtles found stranded there. It is possible that some of the turtles found inshore may have succumbed in the Gulf of Mexico and either drifted inshore or been deposited there intentionally. Similarly, some of the turtles killed in inshore areas, particularly near pass entrances, could have drifted into the Gulf of Mexico and later washed ashore on offshore beaches.

Stranding records demonstrate the importance to Kemp's ridley turtles of coastal waters throughout Texas. Although 35% of the stranded turtles documented from 1980 to 1994 were Kemp's ridley, this species comprised an increasing percentage of the Texas sea turtle strandings since the initiation of the STSSN in 1980, peaking at 48% during 1994.

Kemp's ridleys were found in both inshore and offshore areas, comprising 34% of the offshore strandings and 39% of the inshore strandings. Although more Kemp's ridley turtles were found stranded along the upper Texas coast than along the middle or lower Texas coasts, those located along the middle and lower coasts (exclusive of hatchlings and post-hatchlings) were larger than those along the upper coast. Additionally, all recent records of Kemp's ridley nests and nesting emergences in Texas have been located along the lower Texas coast (Shaver, 1992; Shaver, 1995). Protection of larger sub-adults and adults is critical for efforts to increase the critically endangered Kemp's ridley population (Crouse et al., 1987).

Overall food consumption seemed to be similar between the 1994 and 1983–89 stranded Kemp's ridley turtles. *Nassarius* sp. were found in 14 of the 37 Kemp's ridleys from 1994 and 11 of the 50 from 1983–89. *Nassarius* sp. are scavenging gastropods that feed on dead and decaying crabs and fish (Fotheringham and Bruenmeister, 1989). Plotkin et al. (1993) stated that the presence of *Nassarius acutus* in loggerhead gut contents may indicate that the fish and shrimp consumed by these turtles, which probably could not have captured them alive, were dead when eaten. Presence of these mollusks in stranded Kemp's ridley turtles may also indicate consumption of dead food items, such as those discarded as shrimp trawl by-catch (Shaver, 1991).

Shaver (1991) noted the possibility that Kemp's ridley turtles inhabiting Texas waters may forage on shrimp trawl by-catch, as speculated for loggerhead turtles in Georgia (Shoop and Ruckdeschel, 1982). Kemp's ridley and loggerhead turtles may be attracted to shrimping areas because of the easily obtainable forage available there. This attraction may increase their susceptibility to capture in shrimp trawls that continue to operate in the area.

Numbers of Kemp's ridley turtles may be rising, based on recent increases detected in nesting at Rancho Nuevo, Mexico (Byles[3]). If the Kemp's ridley population is growing, the importance of Texas waters to this species will also increase and these turtles will interact with trawls more frequently. Results of comprehensive conservation programs undertaken on behalf of this species could be negated if large numbers of this species continue to be killed in the marine environment from shrimp trawls and other anthropogenic sources. The wide dispersion of stranding records (for various sized individuals) and nesting records should be considered prior to development of any protection measures for Kemp's ridley turtles in Texas.

Education, enforcement, and research and development activities related to TED's must be continued. Strandings of loggerhead turtles in South Carolina decreased as a result of TED usage (Crowder et al., 1995). Proper installation and usage of TED's, coupled with sustained TED education and enforcement activities, should also reduce sea turtle strandings in Texas. However, if such measures fail to reduce strandings, it may be necessary to temporarily close certain nearshore waters to shrimping to ensure turtle survival. STSSN efforts to document stranded turtles and investigate possible sources of mortality should be expanded so that anthropogenic sources can be identified and reduced.

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Ecotoxicology and Histopathology Conducted in Response to Sea Turtle and Fish Mortalities along the Texas Coast: May–June 1994

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ABSTRACT

Investigative and analytical support was provided during a period of unusual mortalities of various marine species, including protected dolphins, endangered sea turtles, and fish, along the Texas coast during 1994. An ecotoxicological evaluation of the area and an examination of sea turtle and fish carcasses were conducted as part of the emergency response to investigate potential causative factors of a sudden increase in marine species mortalities.

continued

* Laboratory name is now "Center for Coastal Environmental Health and Biomedical Research, National Ocean Service."

Water samples collected from the upper Texas coast between Sabine Pass and Lavaca Bay were analyzed to determine if agricultural runoff was involved in the mortalities. Analytical results indicated that pesticides were not likely causative factors. Fish brain tissue was analyzed for acetylcholinesterase activity but no inhibition was detected, reducing the likelihood that the fish had been exposed to organophosphate or carbamate compounds in the recent past. Necropsies were conducted on sea turtles and fish. Histopathology results did not indicate causes of death of sea turtles or fish, and suggested there was no known common disease factor among any of the animals sampled. Lack of available fresh-dead carcasses severely limited the sample size. Based on the limited number of individual animals analyzed, it appeared that a common causative disease agent was not involved. Because investigating mortalities due to unknown causes requires a scientifically-based process of elimination, it is important in evaluating results of the entire emergency investigation that none of the potential factors examined during this response could be positively linked to observed unusual mortalities.

Introduction

During spring 1994, reported strandings of sea turtles (multiple species) along the Texas and Louisiana coasts increased markedly in comparison to historical databases (Shaver and Teas[1]). The increase in mortality resulted in a total of 527 stranded sea turtles for 1994 relative to a previous record high of 358 dead turtles in 1990 (Shaver, 1998). On 10 May 1994 during the period of increased sea turtle mortality, an unusually large fish kill (Denton et al., 1998) occurred along the same coastal area of Texas. Because it was not known if the mortalities were related and whether threatened and endangered species were being severely impacted by unknown factors, an emergency response was initiated by the National Marine Fisheries Service (NMFS). A forensic investigation was conducted by out-of-state personnel to evaluate the situation and determine the types of samples and analyses needed to examine potential causative factors. Results of ecotoxicology and histopathology are described in this paper. Other aspects of the investigation are presented separately: analysis of samples collected from an algal bloom that was present in the area of mortality (VanDolah et al., 1998), assessment of fish kills (Denton et al., 1998), and fishery interaction issues (Zimmerman[2]).

Methods

The emergency investigation was designed to examine scientifically all possible causative factors, eliminate potential causes based on information obtained during the investigation, and use forensic guidelines, such as chain-of-custody, to protect the integrity of the samples and information collected. Aerial surveys of the region were used to evaluate the geographical extent of the mortalities of marine species and determine the status of agriculture and other industrial activities in the area relative to the deaths. More than 15 water samples and numerous marine animal tissues were collected from the Texas and Louisiana coasts and distributed for analyses, including toxicology, biotoxin screening and bioassay (Van Dolah et al., 1998), physiological biomarkers, and histopathology. Water samples and fish tissues were analyzed for the presence of chemical contamination. The contents of five waste drums that washed ashore in the areas of the fish kill during the on-site sample collection period were also analyzed for priority pollutants.

Water Sampling for Pesticides

Water samples (250 ml) were collected from 17 stations located along the coast of Texas between Sabine Pass and Port Lavaca (Table 1) and analyzed for selected insecticides and herbicides (Table 2) using polyclonal antibody test kits (i.e. Envirogard[R 3], Millipore) and gas chromatography-electron capture and nitrogen-phosphorous detector systems. The specific compounds were selected based upon results from previous investigations conducted along the Texas coast (Colbert et al., In press). Samples were collected from sites where large concentrations of dead fish were observed along Gulf Coast beaches, at estuarine/bay sites adjacent to active agricultural areas, and inland along major agricultural drainage areas.

[1] Shaver, D. (National Biological Service, Padre Island National Seashore, Padre Island, Texas) and W. Teas (National Marine Fisheries Service, Southeast Fisheries Science Center, Miami Laboratory, Miami, Florida). 1994. Personal communications.

[2] Zimmerman, Roger. 1994. NMFS Galveston Laboratory, 4700 Ave. U, Galveston, TX 77551. Personal commun.

[3] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1
Sampling sites for surface water samples along the northern Texas coast.

Site	ECOTOX #	Site description
Site 1	94–112	Hwy 87 detour; N of ICW bridge; drainage ditch (near Stowell)
Site 2	94–113	Agricultural drainage ditch (near Winnie)
Site 3	94–114	Hwy 87 detour; under ICW bridge at Galveston County line; public boat landing
Site 4	94–115	Surf sample; 1 mi past barrier on washed-out Highway 87, northeast of High Island; sea turtle retrieval area
Site 5	94–116	Bay side of Rollover Pass
Site 6	94–117	ICW near Crystal Beach; Siever's Cove
Site 7	94–118	Rice field drainage ditch; 1st one on Hwy 124N (southwest of Beaumont)
Site 8	94–119	Sabine Channel; 200 yd upstream from Hwy 82 bridge
Site 9	94–120	Hwy 73 (south of Beaumont); large creek/drainage ditch; agriculture
Site 10	94–121	Rollover Pass; same as Site 5
Site 11	94–122	San Luis Pass; bay side at bridge around beach curve at south end of Galveston Island
Site 12	94–123	Freeport Channel at Surfside Beach
Site 13	94–124	Colorado River; Bay City at bridge
Site 14	94–125	Just south of Site 13; drainage ditch at rice field
Site 15	94–126	Point Comfort Boat Landing
Site 16	94–127	Formosa Beach on Lavaca Bay; park jetty
Site 17	94–128	Brazos River; under Hwy 322 bridge

Biomarker Analysis: Acetylcholinesterase

Eleven hardhead catfish, *Arius felis*, were collected from various locations where dead fish were observed along washed-out Highway 87 near Sea Rim Park. These fish were collected live, sacrificed, and then frozen and shipped on dry ice to the NMFS Charleston Laboratory for analysis of brain acetylcholinesterase, an enzyme which is often inhibited in aquatic organisms exposed to organophosphate or carbamate insecticides, using methods described by Fulton (1989). Five hardhead catfish were also collected from South Carolina waters to serve as a reference control group.

Chemical Analysis of Beached Waste Barrels

On 17 May 1994 during field sampling, chemical waste barrels were discovered on the beach and beside washed-out Highway 87 near Sea Rim Park. These barrels, which were not present the previous day, were collected by a clean-up contractor (EMTECH of Pasadena, Texas) for the Texas Natural Resources Conservation Commission. EMTECH was contracted to sample these waste barrels to determine whether or not the contents of the barrels might have been factors in the mortalities. The samples were archived, and five subsamples from the archive were analyzed for industrial and priority pollutants (PCB's, pesticides, phenols, cyanide, trace metals, volatile organics, and semi-volatile organics) by a certified analytical laboratory (Pace Environmental, Houston, Texas).

Table 2
Preliminary pesticide analysis of surface water samples from the northern Texas coast. Minus (—) indicates sample was < lower limits of detection (LLOD). Plus (+) and double plus (++) indicates samples were > LLOD and > positive control spike, respectively. For triazine herbicides, ++ indicates > 1.00 μg/L; + indicates ≤1.00 μg/L but ≥0.10 μg/L.

Site	ECOTOX #	Aldicarb	Carbofuran	Triazines
Site 1	94–112	—	—	—
Site 2	94–113	—	—	—
Site 3	94–114	—	—	+
Site 4	94–115	—	—	—
Site 5	94–116	—	—	—
Site 6	94–117	—	—	+
Site 7	94–118	—	—	—
Site 8	94–119	—	—	—
Site 9	94–120	—	—	+
Site 10	94–121	—	—	—
Site 11	94–122	—	—	+
Site 12	94–123	—	—	+
Site 13	94–124	—	—	++
Site 14	94–125	—	—	+
Site 15	94–126	—	—	++
Site 16	94–127	—	—	++
Site 17	94–128	—	—	++

Examination of Fish and Sea Turtles

Though live fish in distress were reported during the fish kill, none had been collected. Live fish from the

same area were collected as soon as possible and kept alive in a tank until shipped. Because sampling of animals occurred as part of an emergency response and logistical problems were encountered, the live fish examined were collected approximately 2 days after the fish kill occurred. Fish including one black drum, *Pogonias cromis*, one pompano, *Trachinotus carolinius*, and approximately 20 hardhead catfish, *Arius felis*, were collected live from the surf at the location of the 10 May 1994 fish kill along washed-out Highway 87. The fish were kept alive in a holding tank over a weekend. The pompano, black drum, and four catfish were sent unfrozen on ice, as requested by the analyst, to the Florida Marine Research Institute for pathology, parasitology, and bacteriology. At the Institute, the pompano, black drum, and two catfish were processed after being on ice approximately 18 hours. Bacteriological swabs were taken from external lesions and kidney of the fish and plated out onto TCBS (thiosulfate-citrate-bile salts-sucrose agar) or TSA (trypticase soy agar) media. Fish were examined by routine diagnostic procedures and samples of gills, liver, kidney, spleen, pancreas, and gall bladder were processed for histopathology. Tissues were fixed in 5% paraformaldehyde in 0.1M phosphate buffer, dehydrated in a graded ethanol series, and embedded in JB-4 glycol methacrylate resin. Sections were cut to 3.5 μm on an LKB 2218 Historange microtome and were stained in Weigert's hematoxylin and eosin (H & E), or in thionin stain adapted to glycol methacrylate (Nagle and Quintero-Hunter)[4]. Brain tissue from each fish was wrapped in aluminum foil and immediately stored frozen. The remains of the examined fish and two intact catfish were archived. Formalin-fixed gill and liver tissues from five hardhead catfish were examined by another researcher. The remaining fish were archived.

The majority of sea turtles that had stranded dead since the beginning of the event were collected and stored frozen by the NMFS Galveston Laboratory, thus standard histopathology was not possible. Many of the turtles that stranded before and during the emergency response were too decomposed to yield much information indicative of cause of death. The decomposed turtles were identified to species, measured, and documented for baseline stranding information. Five sea turtles were suitable for histopathology. Tissue was collected in neutral buffered formalin during necropsy. Fixed tissue was sent to North Carolina State University for histopathologic evaluation. Tissues were embedded in paraffin and sectioned and stained with hematoxylin and eosin.

[4] Nagle, P., and I. Quintero-Hunter. 1994. Florida Department of Environmental Protection, Florida Marine Research Institute, 100 8th Avenue, S.E., St. Petersburg, FL 33701. Unpubl. manuscr.

Results

Pesticide Screening

During May 1994, aerial and ground surveys of the area along the upper Texas coast showed that many agricultural crops were in early growing stages. Crop dusters and spraying equipment were observed in use and documented. The rice fields in most areas recently had been flooded for harvest. Surface water samples collected at 17 sites between Sabine Pass and Lavaca Bay were screened for two insecticides (carbofuran and aldicarb) and one class of herbicides (triazines) using the Envirogard^R polyclonal antibody assay system (Table 2). None of the samples were positive for either insecticide. Ten of 17 samples were positive for triazine herbicides. Two years of follow-up research, subsequent to the 1992 event involving bottlenose dolphins along the mid-Texas coast, demonstrated that triazine herbicides could be detected in some estuarine/bay systems during most months of the year (Pennington, 1996).

Four of the 10 positive samples contained triazine levels >1.0 ppb. These four samples were also analyzed by gas chromotography for the presence of four additional insecticides (azinphosmethyl, endosulfan, chlorpyrifos, and fenvalerate) commonly used in the area. The results of the analyses were negative in the four samples for each of the four insecticides.

Water samples obtained for pesticide screening were collected immediately following rainfall in areas where agricultural runoff would most likely occur. Results of the pesticide screening assays were positive only for atrazine, a triazine herbicide. No other pesticides were detected. Because samples were collected under optimum conditions to detect runoff chemicals, it is unlikely that agricultural runoff contributed to the unusual mortalities.

Brain Acetylcholinesterase Activity in Hardhead Catfish

Under certain conditions, activity levels of the enzyme acetylcholinesterase (AChE) measured in brain tissue can be used as a forensic tool to indicate whether or not an animal has been exposed to cholinesterase-inhibiting compounds such as carbamate and organophosphate insecticides (Coppage and Braidech, 1976). Preliminary results of brain AChE, measured in tissue samples removed from 11 hardhead catfish, ranged from 13.21 to 20.20 nmol mg tissue^{-1} min^{-1} (Table 3). The mean brain AChE activity in these fish was 16.61 nmol mg tissue^{-1} min^{-1}. Brain AChE activity in the five fish collected from South Carolina waters ranged from 15.97 to 18.59 nmol mg tissue^{-1}min^{-1}. Mean brain AChE

activity in these fish was 17.25 nmol mg tissue^{-1} min^{-1}. There was no significant ($p = 0.57$) difference in brain AChE activity between the Texas and South Carolina fish. These results indicate there were no biologically significant levels of brain AChE inhibition in the Texas catfish. This greatly reduces the likelihood that the Texas catfish were exposed to cholinesterase-inhibiting agents in the recent past.

Analysis of Beached Chemical Barrels

The results of chemical analyses conducted on samples from five waste barrels are shown in Table 4. One drum may have contained gasoline and a pesticide residue. Most of the other components detected were thought to be byproducts of the breakdown corrosion in the metal barrels. Results of the analyses indicated that the contents of the drums sampled were most likely not involved in the marine animal mortalities.

Examination of Fish Tissues

All of the fish that had been collected live exhibited hemorrhagic fins and epidermal cell loss on the fins. No inflammation was observed accompanying the epidermal loss and hemorrhage. Gills showed degeneration and post-mortem change, most likely due to the shipment period of approximately 18 hours. The livers of the fish examined at one facility were found to be normal.

Table 3

Brain AChE activity measured in hardhead catfish brain tissue.

ID number	Description	Brain AChE activity (nmol mg^{-1} min^{-1})
UM 1-7	Catfish (Charleston, SC)	18.59
UM 1-11 (1)	Catfish (Charleston, SC)	15.97
UM 1-11 (2)	Catfish (Charleston, SC)	16.82
UM 1-11 (3)	Catfish (Charleston, SC)	17.20
UM 1-11 (4)	Catfish (Charleston, SC)	17.66
UM 1-2 (3)	Catfish (Texas)	13.21
UM 1-2 (2)	Catfish (Texas)	13.59
UM 1-2 (4)	Catfish (Texas)	14.90
UM 1-4 (4)	Catfish (Texas)	20.20
UM 1-4 (5)	Catfish (Texas)	14.28
UM 1-3 (2)	Catfish (Texas)	15.90
UM 1-3 (6)	Catfish (Texas)	17.89
UM 1-3 (9)	Catfish (Texas)	18.28
UM 1-3 (8)	Catfish (Texas)	18.66
UM 1-3 (7)	Catfish (Texas)	18.20
UM 1-3 (10)	Catfish (Texas)	17.59

Table 4

Summary of chemical analysis of waste drums washed ashore along the Bolivar peninsula north of Galveston, Texas. Only compounds with concentration > LLOD are listed.

Barrel	Contaminant	Conc. (μg/Kg)	Comment
Crystal Beach #1	Ni	4,000	No organics[1], primarily rust and corrosion.
	Zn	5,000	
	Phenolics	20,000	
Pelican Pier #1	Benzene	5,200,000	This barrel contained gasoline and some BHC.
	Ethylbenzene	5,800,000	
	Toluene	18,000,000	
	Naphthalene	53,000	
	Beta BHC	1.7	
	Zn	1,000	
	Phenolics	29,000	
Beau #4	Cu	4,000	No organics[1], primarily corrosion
	Pb	65,000	
	Zn	240,000	
	Phenolics	13,000	
Beau #12	Cu	19,000	No organics[1], primarily corrosion
	Zn	130,000	
	Phenolics	4,500	
Beau #13	Zn	160,000	No organics*, primarily corrosion
	Phenolics	2,600	

[1] Organics in this case refers to pesticides, PCB, herbicides, fuel oils, and related compounds.

The following conditions were observed in four other fish examined:

Hardhead catfish #1: Bacterial isolates from the anal fin included two *Aeromonas* spp., *Vibrio* sp., and presumptive *Photobacterium angustum*. Petechiae present on the skin may have been associated with infestations of *Caligus haemulonis*, a copepod. *Aeromonas* sp. was also isolated from the kidney. An inflammatory response with marked neutrophilia and karyorrhexis was observed in the interstitial tissue of the kidney. The liver appeared to be congested and was vacuolated in both defined areas of the tissue as well as in individual hepatocytes. Analysis of the spleen determined that melanomacrophage centers were large with an increase in yellowish-brown pigmentation (hemosiderin), and many nuclei were present (possibly due to increased lysis of erythrocytes, but erythrocytes in blood vessels were normal). There was no obvious increase in erythroblasts which might have been present if associated with hemolysis due to a biological toxin from bacteria (*Vibrio* sp.) or dinoflagellates.

Hardhead catfish #2: The edge of the spleen tissue was degenerate due to postmortem change. The melanomacrophage centers were large, with an increase in yellowish-brown pigmentation and many nuclei. Erythrocytes in blood vessels were normal with no obvious increase in erythroblasts. Kidney tissue was beginning to degenerate, with obvious detached vacuolated epithelial cells from the kidney tubules. Interstitial tissues contained some karyorrhectic cells and neutrophils, possibly indicating early inflammatory response and necrosis. Bacterial growth was negative.

Pompano: Gills showed some degeneration of the secondary lamellae, and low level infestation by the monogenetic trematode *Bicotylophora trachinoti*. There was also a low level infestation of epitheliocystis. There were myxosporean spores in the gall bladder bile. Vacuolated epithelial cells were detached from the basement membrane of the kidney tubules, suggesting degeneration. Interstitial tissue was vacuolate with some karyorrhectic cells or neutrophils, necrosis, and large numbers of eosinophilic granulocytes. Bacterial growth was negative. Postmortem degeneration was observed in spleen samples. Many granulomas were present. Liver tissue was congested, or filled with red blood cells, and had vacuolation both in defined areas of the tissue and in individual hepatocytes. Some karyorrhectic cells were present in blood vessels. The pancreas was normal.

Black drum: Bacterial isolates from the caudal fin included two *Aeromonas* spp. and two *Vibrio* spp. Gills were lightly infested with monogenetic trematodes, *Microcotyle pogoniae*. *Aeromonas* sp. was also isolated from the kidney. An inflammatory response with marked neutrophilia and karyorrhexis was observed in the interstitial tissue of the kidney. The spleen was hemorrhagic, with karryorhectic cells in interstitial tissue, and was very congested and vacuolate. The liver hepatocytes were vacuolar with some karyorrhectic cells in blood vessels and a few melanomacrophage centers. The pancreas was beginning to degenerate.

The hemorrhagic condition of the fish fins is a characteristic generally associated with stress, and not usually used as a diagnostic factor. It could not be determined whether this characteristic resulted during capture and handling of the fish, or whether it developed prior to capture. The reddish fin condition was observed to worsen during the holding period prior to shipment and was photographically documented.

Because the fish were placed in a transfer tank for transport to the laboratory, kept in an artificial system for 3 days, and examined approximately 18 hours after time of death, bacteriological results are not necessarily considered to be representative of the condition of the fish in their natural environment. The bacterial isolates were indicative of secondary opportunists that may have infected the fish after other factors had stressed the fish. The time frame between collection and examination would most likely mask any factors causing an acute pathological response, however, some disease agents might still have been detectable had they been present.

Histopathological Analysis of Sea Turtle Tissues

There were no disease-related histologic lesions seen in any turtle tissues that indicated a cause of death of the four Kemp's ridley, *Lepidochelys kempi*, and one loggerhead, *Caretta caretta*, submitted. Tissue preservation was good and histologic detail was maintained in all samples, so any abnormal conditions present should have been apparent.

One Kemp's ridley turtle (UM1-12) had minor to moderate pulmonary edema, with little associated inflammatory response. Because this animal had been placed on a respirator during its clinical care, the lesion was attributed to the therapy. A second Kemp's ridley turtle (UM1-15(2)) also had mild edema and an accumulation of mucus within the airway, indicative of respiratory tract irritation but not attributable to a specific cause. Congestion and edema were found in the lung of a loggerhead turtle (UM1-15(1), SCC271) and a Kemp's ridley (UM1-15(3)). The liver of one turtle (UM1-15(3)) had a focus of infarction at the periphery of the lobe and associated heterophil infiltration.

Overall, there was no specific lesion indicative of a cause of death in the sea turtles examined. The most common lesion occurred in the lungs, but edema was generally mild. Death by drowning could not be determined as the cause of death because control tissue from

animals that were known to have died by drowning was not available.

Discussion and Conclusions

Histopathological results indicated that there were no known common disease factors or other common cause of death among the turtles and fish sampled. To determine the cause of the overall increase in sea turtle and fish mortality during the period, much more extensive diagnostic effort would have been necessary at the beginning of the unusual mortality period so that a larger number of recently dead specimens could have been obtained. Collection of tissue for histologic and ultrastructural examination must be performed promptly after an animal dies since autolysis or freezing artifacts seriously impair an accurate histologic diagnosis. Such a collection was conducted only near the end of the investigation.

There is no evidence based on examination of fish and sea turtle tissues collected for this study to indicate that the fish kill events and sea turtle mortalities were related. Reports of live fish in distress in the surf may support the hypothesis that the 10 May 1994 fish kill occurred relatively close to shore. The majority of fish collected for examination did not exhibit active signs of distress but were collected at least 24 hours after the major fish kill occurred. If fish were exposed to acute primary stressors such as toxic algal blooms or low dissolved oxygen, then the pathological and bacteriological changes observed in the fish could be explained as a secondary response. Such primary stressors could lead to secondary bacterial infections during the time course of the investigative response to the fish kill. However, no obvious causative agents were detected during initial field observations. The potential for exposure to a natural toxin or impact by some environmental contaminant cannot be completely eliminated as a factor in the fish kill, and results of water analyses for presence of biotoxins may indicate one potential causative factor (VanDolah et al., 1998).

The responses to the unusual 1992 and 1994 mortality events involving coastal and marine species along the Texas coast underscore the value of the integrated investigative approach of which these analyses were a part. It also indicates the need to develop further communications and funding for analytical support of such events. The two mortality events have created an interest among numerous local, state, and federal agencies and institutions in responding to such crises along the coast of the Gulf of Mexico as early, and as effectively, as possible when future unusual mortalities of marine species are detected. Such a planned team approach composed of people already present in an area should help prevent some of the problems encountered in this response.

Acknowledgments

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Gross Necropsy Results of Sea Turtles Stranded on the Upper Texas and Western Louisiana Coasts, 1 January–31 December 1994

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ABSTRACT

Necropsies were conducted on 194 of the 284 sea turtles stranded on the upper Texas and western Louisiana in 1994. The species necropsied included 167 Kemp's ridley sea turtles, *Lepidochelys kempii* (86.1% of total); 20 loggerheads, *Caretta caretta* (10.3%); 6 greens, *Chelonia mydas* (3.1%); and 1 leatherback, *Dermochelys coriacea* (0.5%). External injuries or trauma were recorded for 28% of the necropsied turtles. The sex ratio of the necropsied turtles was 1:1. Gastrointestinal tracts of stranded sea turtles contained primarily fish and crabs. Fish hooks, plastic beads and bags, and balloons were also found in the stomach and intestinal tracts. Possible causes of death identified for sea turtles stranded during 1994 include ingestion of fish hooks, congenital deformities, boat propeller injuries, and entanglement in fishing gear. A primary causative agent could not be determined.

Introduction

The National Marine Fisheries Service (NMFS) Galveston Laboratory has participated in the Sea Turtle Stranding and Salvage Network (STSSN), conducting systematic surveys since 1986 (Heinley et al., 1988; Duronsolet et al., 1991). The Galveston Laboratory's involvement now includes responding to call-in reports, documenting sea turtle strandings, providing systematic surveys of the upper Texas and western Louisiana coasts, conducting necropsies on dead stranded sea turtles, and rehabilitation of live stranded sea turtles.

During spring of 1994, strandings of sea turtles (Shaver[1]) and marine mammals were at record levels. Over 200 marine mammals, primarily bottle-nosed dolphins, *Tursiops truncatus*, were stranded dead between February and May 1994 on the upper Texas coast (Haubold[2]). In April marine mammal strandings began to decline, and sea turtle strandings increased. Large numbers of hardhead, *Aerius felis*, and gafftopsail catfishes, *Bagre marinus*, were also reported washed ashore along the upper Texas coast concurrent with sea turtle strandings.

The purpose of this paper is to report the results of necropsies from 194 sea turtles which stranded during 1994.

Methods

The turtle's condition was immediately assessed to determine its disposition (Table 1). Dead stranded sea turtles were brought to the Galveston Laboratory and, depending on the state of decomposition, either frozen for gross necropsy at a later date or necropsied immediately. Live stranded sea turtles were retrieved and brought to the Galveston Laboratory or University of Texas Medical Branch (UTMB), Galveston, for rehabilitation.

Of the 284 sea turtles reported stranded along the upper Texas and western Louisiana coasts in 1994, 194 were necropsied as described by Rainey (1981) and Wolke and George (1981). Complete necropsies (including histology and pathology) were performed on

[1] Shaver, Donna. 1994. Padre Island National Seashore, Midwest Science Center, National Biological Service, 9405 S. Padre Island Dr., Corpus Christi, TX 78418. Personal commun.

[2] Haubold, Elsa. 1994. Texas Marine Mammal Stranding Network, 4700 Ave. U, Galveston, TX 77551. Personal commun.

Table 1
Description of carcass condition and appropriate tissue samples collected.

Condition	Disposition	Description	Tissues collected
Live stranded, died at holding facility	Necropsy immediately	Extremely fresh, no postmortem changes	Complete suite of histology and toxicology samples, blood work-up, GI tract analyses, determine sex
Fresh dead, no bloating	Placed on ice, necropsy immediately	Stranded dead, no bloating	Histology and toxicology samples, GI tract analyses, determine sex
Moderately decomposed	Frozen, for gross necropsy	Some bloating, internal organs identifiable	Toxicology, GI tract analyses, determine sex
Severely decomposed	Frozen, for gross necropsy	Organs decomposing, not easily identifiable	GI tract (when possible), determine sex (when possible)

very fresh carcasses (minimal postmortem deterioration) and on any live stranded animals that later died.

Carcasses that were severely decomposed were of little or no use for histology or pathology and were frozen for gross examination when time permitted. Toxicology samples, however, were collected from certain of these carcasses (Table 2), and natural history data (i.e., feeding habits, sex ratios) was collected when possible.

Stranded turtles were initially examined for obvious signs of external trauma. Necropsies included observations of internal organs, qualitative analysis of gastrointestinal tracts, and visual examination of gonads to determine sex when possible. Tissues for histological analysis were preserved in 10% neutral buffered formalin and samples for toxicological analyses were frozen. Histological samples from the first live stranded Kemp's ridley, which died later, were taken to UTMB for analysis. All other toxicological and histological samples were shipped to the NMFS Charleston Laboratory, Charleston, S.C., for analysis.

Table 2
Frequency of tissue types collected.

	Kemp's ridley	Loggerhead	Green	Leatherback
Blood chemistry	3	2	0	0
Histology/pathology	8	2	0	0
Toxicology	9	2	0	0
Gross necropsy only	158	18	6	1

Results

Individuals of the five species of sea turtles occurring in the Gulf of Mexico were stranded along the upper Texas and western Louisiana coasts during 1994 (Table 3). Species composition of the necropsied turtles was 86.1% Kemp's ridleys (167), 10.3% loggerheads (20), 3.1% greens (6), and 0.5% leatherbacks (1).

Live Strandings

Nine Kemp's ridleys, three loggerheads, one green, and one hawksbill, *Eretmochelys imbricata*, were stranded alive. Five of the Kemp's ridleys and the three loggerheads died within 72 hours of being retrieved for rehabilitation. The seven sea turtles which died within 72 hours of being brought to the NMFS Galveston Laboratory had the greatest potential for identifying possible causes of the spring 1994 stranding event (Stabenau[3]). Two Kemp's ridley, one hawksbill, and one green showed signs of external trauma. All four were successfully rehabilitated and certified for release by veterinarians.

Two Kemp's ridleys survived more than 72 hours before dying. Necropsy revealed that both had abnormal development of the lungs. Defects causing inefficient gas exchange were likely contributors to the demise of these two animals.

External Injuries

A variety of external injuries were reported for dead stranded turtles but causes of the injuries could not be determined. One hundred twelve (72.3%) of the 155

[3] Stabenau, E. K. 1994. Blood chemistry profiles of live-stranded and captive reared Kemp's ridley and loggerhead sea turtles. Unpubl. manuscr.

Table 3
Live stranded and dead stranded sea turtles along the upper Texas and western Louisiana coasts during 1994.

Species	Live/died at holding facility	Live/successfully rehabilitated	Dead	Total stranded	Total necropsied
Kemp's ridley	7	2	216	225	167
Loggerhead	3	0	47	50	20
Green	0	1	6	7	6
Leatherback	0	0	1	1	1
Hawksbill	0	1	0	1	0
Total	10	4	271	284	194

necropsied sea turtles had no external injuries. Seventeen (11.0%) were missing at least one appendage (including head and/or flippers). According to Richard Henderson[4], appendages of two of these turtles may have been removed mechanically, based on straight edge cuts on the anterior margins of the carapace. It is not possible, however, to determine whether this caused the turtles' deaths or if it occurred post mortem. Due to advanced decomposition, we were unable to determine whether appendages were cut off at sea or removed by scavengers on the remaining 15 carcasses. Nineteen (12.3%) carcasses had damage to the carapace consistent with injuries caused by boat propellers. Six (3.9%) had miscellaneous damage to the head and/or flippers but again we could not determine the cause of these injuries. One (0.7%) Kemp's ridley was entangled in monofilament fishing line which was wrapped around its head and all four flippers. The extent of the entanglement would likely have impeded ability of the turtle to swim and surface for air, resulting in asphyxiation. It is possible, however, that the turtle became entangled after death.

Sex Ratio

The observed sex ratio for necropsied Kemp's ridley sea turtles in 1994 was 41:48. Fifty-seven Kemp's ridley carcasses were too severely decomposed to determine gender. The ratio of females to males varied with size, ranging from 1:1.5 for Kemp's ridleys 30.0–39.9 cm straight carapace length (SCL) to 2.5:1 for Kemp's ridleys greater than or equal to 50.0 cm (Table 4). Of the 20 loggerheads necropsied, eight were females; the gender of 11 could not be determined. Gender could not be determined for the green turtles or the sole leatherback.

[4] Henderson, Richard. 1994. Galveston Island Veterinary Clinic, Galveston, TX. Personal commun.

Table 4
Sex and size distribution of necropsied sea turtles stranded during spring 1994.

Straight carapace length (cm)	Kemp's ridley		Loggerhead	
	F	M	F	M
20.0–29.9	11	13	0	0
30.0–39.9	16	24	0	0
40.0–49.9	9	9	2	0
50.0–59.9	4	2	6	1
>59.9	1	0		
Total	41	48	8	1

Gastrointestinal Tract

The gastrointestinal (GI) tracts of necropsied turtles were examined grossly for food items, debris, and possible blockages. Ten of the 176 Kemp's ridleys examined contained no food or debris in the GI tract, and 45 were too severely decomposed to determine GI tract contents. Nine of the GI tracts were collected in their entirety and sent to the NMFS Charleston Laboratory for toxicological analysis. The most commonly occurring food item for all sea turtle species combined was fish parts (occurring in 40.2% of the turtles), including bones of hardhead catfish as well as other unidentified fish species. Crab parts, including blue crabs, *Callinectes* spp., stone crabs, *Menippe mercenaria*, purse crabs, *Persephona mediterranea*, and unidentified crabs, occurred in 38.1% of the necropsied Kemp's ridleys. Seven of the Kemp's ridleys contained *Nassarius* spp. (Table 5), a small gastropod known to scavenge, suggesting that the turtles may have been feeding on dead and decaying organisms.

Fish hooks were found in five Kemp's ridleys. One Kemp's ridley GI tract contained parts of crabs, a bird, as well as a fish hook embedded in the esophagus. An abscess 4 cm in diameter was associated with the fish

Table 5
Frequency of items observed in the gastrointestinal tracts of sea turtles necropsied during 1994, by species.

Food item	Kemp's ridley	Loggerhead	Green	Total
Fish	74	4	0	78
Crab	70	4	0	74
Plastic	9	1	2	12
Plant	8	1	4	13
Fish hook	5	0	0	5
Shell hash	6	0	0	6
Nassarius spp.	7	0	0	7
Moon snails	3	1	0	4
Bird	1	0	0	1
Tube worms	1	1	0	2
Monofilament line	1	0	0	1
Sea anemones	0	1	0	1
Goose neck barnacles	1	0	0	1
Empty	6	1	0	8[1]

[1] Includes the sole leatherback necropsied.

hook. Due to advanced decomposition, however, it was impossible to determine whether this was the cause of death. Another Kemp's ridley had a fish hook in the intestine; there was no sign that it had perforated the intestine, stomach, or esophagus. Fish hooks were also found embedded in the esophagus of three additional Kemp's ridleys. Given decomposition of these three animals no abscess was noted. *Sargassum* spp. was identified in four of the Kemp's ridley GI tracts. Plastic debris occurred in ten of the specimens, although it did not appear to cause any blockage or trauma.

The GI tract of four loggerheads contained fish bones. The GI tracts of four of the green sea turtles contained unknown plant material; one GI tract was empty. Two of the green's GI tracts also contained plastic. The GI tract of the leatherback was empty.

Discussion

The cause of death could not be determined by gross necropsy of any of the sea turtles examined during 1994. Although complete necropsies were not performed on all turtles due to the state of decomposition of the carcass, gross necropsies on such carcasses can still yield valuable information. Occasionally a probable cause or contributing cause of death (particularly if there is a blockage or perforation of the GI tract) can be determined. Moreover, possible factors contributing to death can be ruled out.

External Injuries

Although external injuries were reported in 27.7% of the sea turtles necropsied, it was not possible to attribute deaths to these injuries. Heinley et al. (1988) reported mutilation rates of 26% and 60% in stranded sea turtle carcasses collected along the upper Texas coast, in 1986 and 1987, respectively. They listed several possible causes of mutilations, i.e. boat propellers, post mortem scavengers, shark attack, and human induced injury (at sea or on the beach). We were unable to determine whether the injuries observed resulted in death or occurred post-mortem.

Sex Ratio

Sex ratios of 3:1 for Kemp's ridleys and 2:1 for loggerheads have been reported from necropsies of 257 stranded sea turtles collected in the western Gulf of Mexico (Stabenau[5]). Our investigation failed to show dominance of females in sex ratios of Kemp's ridleys and loggerheads. Dave Owens[6] has suggested that juvenile female sea turtle gonads may decompose faster, becoming harder to identify than the more densely-packed juvenile male gonads. Varying decomposition rates of gonadal tissue will yield biased sex ratios and could account for the 1:1 sex ratio in this study.

Gastrointestinal Contents

Debris (including fish hooks and plastic) was found in 9.0% of the necropsied sea turtles; in no case, however, was it apparent that debris contributed to the demise of a turtle. Stanley et al. (1988) reported debris in 26.8% of turtles necropsied during 1986 and 40.6% of those necropsied in 1987.

Gross analyses of GI tract contents of Kemp's ridleys stranded in 1994 were comparable with the findings of Shaver (1991) who analyzed the contents of 100 Kemp's ridleys. She reported that juveniles fed on crabs and fish, appearing to be more opportunistic feeders than adults. Kemp's ridleys have been observed following shrimp boats and feeding on discarded by-catch (Carpenter[7]). The analyses of GI tract contents for loggerhead sea turtles proved consistent with Plotkin et al. (1993), with loggerheads feeding primarily on crab and fish.

[5] Stabenau, Erich K. 1994. NMFS Galveston Laboratory, 4700 Ave. U, Galveston, TX 77551. Personal commun.

[6] Owens, Dave. 1994. Texas A&M University, Dept. of Biology, College Station, TX 77843. Personal commun.

[7] Carpenter, James. 1993. NMFS Galveston Laboratory, 4700 Ave. U, Galveston, TX 77551. Personal commun.

The results of the necropsies performed on stranded sea turtles collected in 1994 did not indicate any primary causative agent for the strandings along the upper Texas and western Louisiana coasts during 1994.

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